

**FY02**  
**NIH Extramural Support**  
**in Bacteriology Research**

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R21AA013434-01  
**Program Director:** LUCAS, DIANE  
**Principal Investigator:** GENTRY-NIELSEN, MARTHA J PHD  
**Title:** Smoking and Ethanol-Induced Defects in Pneumonia Defense  
**Institution:** CREIGHTON UNIVERSITY OMAHA, NE  
**Project Period:** 2001/12/01-2004/11/30

DESCRIPTION (provided by applicant): The goal of this application is to use a novel rat model to study the compounding effects of cigarette smoke and alcohol abuse on susceptibility to severe pneumococcal pneumonia. Pneumonia is a major cause of morbidity and mortality in alcoholics, and the pneumococcus is the most common bacterial cause. Alcoholics have a higher incidence of pneumococcal pneumonia and they have a greater likelihood of developing bacteremia, which increases their mortality rate. Although scientists have studied the deleterious effects of ethanol ingestion on resistance to infectious diseases for years, the concurrent effects of smoking have been ignored. Because 80-90% of alcoholics smoke and >50% of multi-pack/day smokers are alcohol dependent, it is imperative to consider the additional effects of smoking when studying alcohol-induced defects in host defense against respiratory infections. Our hypothesis is that smoking exacerbates the detrimental effects of ethanol ingestion on host defense mechanisms critical for protection against lethal pneumococcal pneumonia. To test this hypothesis, rats will be exposed twice daily to cigarette smoke or room air in whole body chambers. Half of the rats also will be fed ethanol in a liquid diet. In Specific Aim 1, the rats will be infected intranasally, and the numbers of pneumococci reaching their lungs will be quantified by plate-counts. Movement of the organisms into the lungs will be correlated with alterations in the ciliary beat frequency of the rats' tracheal epithelial cells. In Specific Aim 2, novel in vitro assays will be used to determine the effects of ethanol ingestion, with and without smoke exposure, on the ability of the rats' pulmonary neutrophils to phagocytose and kill pneumococci. In Specific Aim 3, quantitative blood cultures will be used to determine the separate and combined effects of smoke exposure and ethanol ingestion on bacteremia development after establishment of pneumonia. Bacteremia will be correlated with mortality for 10-days post-infection with the use of a hypothermia model to predict death and determine the appropriate time for euthanasia.

**Grant:** 1R55AG021097-01  
**Program Director:** FULDNER, REBECCA A.  
**Principal Investigator:** TURNER, JOANNE PHD  
**Title:** CD8 T cells and immunity to tuberculosis in old mice  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 2002/09/30-2003/06/30

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 that specifically targets an aging immune system 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 rapid 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. Using the low-dose aerosol infection model of tuberculosis we will characterize this CD8 T cell population further by determining the signals that rapidly recruit it to the lungs, and the mechanism by which it mediates early resistance to infection. Finally, we will use vaccine strategies known to elicit antigen specific CD8 T cell responses to mycobacterial antigens to see if these cells can then be detected within the CD8 cell population recruited into the lungs of old mice after infection with *M. tuberculosis*. Studies will be carried out in a new BSL-3 facility at Colorado State University and will use old wild type and gene-disrupted mice from our existing in-house aging mouse colonies. The technical approaches in the proposed studies will use a combination of flow cytometry, FACS cell sorting, immunohistochemical staining, and real-time PCR, to address the proposed Aims.

**Grant:** 1U01AG021406-01  
**Program Director:** FULDNER, REBECCA A.  
**Principal Investigator:** NOVAK, M JOHN DDOT DENTISTRY  
**Title:** Aging: Effects on Infection, Inflammation and Disease  
**Institution:** UNIVERSITY OF KENTUCKY LEXINGTON, KY  
**Project Period:** 2002/09/01-2006/08/31

DESCRIPTION (provided by applicant): This proposal is a collaborative submission from the University of Kentucky, the University of Maryland, and the Nutritional and Molecular Physiology Unit of the Laboratory of Neurosciences of the National Institute on Aging. The proposed studies will build on existing data obtained from Rhesus monkeys being maintained on calorie restricted and normal diets by the NIA, and will provide a biologic basis for our preliminary observations of altered inflammatory responses in calorie restricted animals. This proposal focuses on utilizing the oral cavity as a model system to examine the impact of aging on host-bacterial interactions as they relate to microbial colonization of mucosal surfaces, the induction and regulation of inflammatory/immune responses, and the pathologic destruction of host tissues that may result from these interactions. The aims of this study will test the following contrasting scientific hypotheses: (a) that caloric restriction (CR) reduces clinical inflammation by affecting the pathogenicity of microbial plaque, and that these changes are due to shifts in the proportions and/or clonal type of the constituent pathogenic and non-pathogenic microorganisms; (b) that CR alters the clinical manifestation of inflammation through an effect on innate immune mechanisms through increased pro-inflammatory molecule release; and/or (c) that CR alters the clinical manifestation of inflammation through an effect on innate immune mechanisms by regulating the release of anti-inflammatory molecules. Aim 1 is a cross-sectional retrospective study to determine the effects of a long-term calorie restricted diet on the progression of naturally occurring inflammatory periodontal disease in CR and non-CR Rhesus monkeys. The effects of CR on clinical inflammation, microbial colonization of mucosal surfaces, and pro-inflammatory and anti-inflammatory mechanisms will be evaluated. Aim 2 is a longitudinal prospective study to determine the effects of a calorie-restricted diet on the clinical, microbiological, and host responses observed during experimental ligature-induced periodontitis in the same CR and non-CR Rhesus monkeys. These studies will provide information on the effects of CR on the kinetics of clinical, microbiologic, and inflammatory changes at mucosal sites. The significance of these studies lies in our capability to use nonhuman primates and the oral cavity to evaluate the efficacy of CR as a means to regulate infection, inflammation, and inflammatory disease. The long-term implications are that diet control may be considered as an effective public healthcare measure for improving the oral and general health and welfare of the population as a whole.

**Grant:** 2R01AI018188-22

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

**Principal Investigator:** MUNFORD, ROBERT S MD INTERNAL  
MED:INFECTIOUS DISEASE

**Title:** INTERACTIONS OF ENDOTOXIN WITH PLASMA AND CELLS

**Institution:** UNIVERSITY OF TEXAS SW MED DALLAS, TX  
CTR/DALLAS

**Project Period:** 1981/08/01-2007/04/30

DESCRIPTION (provided by applicant): Gram-negative bacteria are prominent members of the microbial flora of all animals. When they invade into tissue, often through a break in the epithelium, they are quickly countered by the body's innate host defenses. Much of this beneficial response is triggered by a bacterial cell wall lipopolysaccharide (LPS) that, because it can also induce lethal reactions, has earned the name "endotoxin." The long-term goal of our research has been to learn how animals inactivate (detoxify) LPS. During the next funding period, we hope to define the biological function(s) of an animal enzyme, acyloxyacyl hydrolase (AOAH), that selectively cleaves the secondary fatty acyl chains from the lipid A region of LPS. Although enzymatically-deacylated LPS is inactive in several assay systems, suggesting that AOAH should detoxify LPS in vivo, the precise contribution of the enzyme to LPS detoxification is uncertain and the enzyme may have functions unrelated to its ability to deacylate LPS. The proposed research builds on 3 recent developments in our lab: the discovery that AOAH is present in immature dendritic cells and can deacylate E. coli that the cells ingest, the finding that AOAH is abundantly produced in the proximal tubule cells of the kidney and secreted into urine, and the targeted disruption of the AOAH gene to produce AOAH "knockout" mice. Our Specific Aims are (1) to determine the role of LPS deacylation in LPS inactivation, in vitro and in vivo, (2) to find out the function(s) of acyloxyacyl hydrolase (AOAH) in the kidney, and (3) to determine the role of LPS deacylation in the presentation of LPS and bacterial outer membrane proteins to B and T cells. Finding out the functions of this highly conserved enzyme should shed new light on how animals control their inflammatory and immune reactions to gram-negative bacteria.

**Grant:** 2R01AI019018-19A2  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** CROSA, JORGE H PHD  
CHEMISTRY:CHEMISTRY-  
UNSPEC  
**Title:** Iron Uptake as a Virulence Factor in Pathogenic Vibrios  
**Institution:** OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR  
**Project Period:** 1982/04/01-2007/02/28

DESCRIPTION (provided by the applicant): The long-term goal of this research is to dissect the complex mechanism of regulation of virulence-associated gene expression in the pathogen *Vibrio anguillarum*. The plasmid-mediated iron uptake system is a major component of this bacterium's virulence repertoire, playing an essential role in the host-bacteria interaction leading to systemic infection and mortality. This system serves as an excellent model for the study of the role that iron scavenging plays in systemic infections of humans since the pathogenesis of this infection shows striking similarities to human septicemic disease caused by other vibrios. We have characterized the essential components of this iron uptake system: the siderophore anguibactin, transport proteins, and regulatory elements controlling its synthesis and transport. In this system we identified an iron transport-biosynthesis operon whose expression is controlled by the positive regulators AngR and TAF that act at regions that include the promoter of this operon. We also characterized two antisense RNAs, RNAalpha and RNAbeta, that act post-transcriptionally in the negative control of the iron transport-biosynthesis operon. RNAalpha acts in the control of expression at the fatA-farB intergenic region and RNA beta acts as an attenuator of the expression of angR as compared to upstream genes in the operon. Therefore, we propose: (1) to characterize the roles of the two antisense RNAs, RNAalpha and RNA beta, as negative regulators of gene expression and to investigate the molecular and structural parameters that affect their stability, activity and influence on virulence. (2) To characterize the role of the positive regulators, AngR and TAFr, in the expression of the iron transport-biosynthesis operon, anguibactin production, and virulence. We intend to generate site-directed and deletion mutants in the angR gene and in the TAFr region in order to dissect the genes that are essential for regulation and virulence. The specific mechanisms by which these components govern the pathogen-host interaction will be further elucidated. Dissection of the molecular interactions underlying the expression of the iron uptake system in this pathogen will serve as a paradigm to the exploration of new avenues in understanding the process of systemic human infection of a bacterial etiology. In the long term, our findings will aid in the development of measures for the control of septicemic diseases.

**Grant:** 2R01AI019296-21

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

**Principal Investigator:** SIMON, MELVIN I PHD  
BIOCHEMISTRY:NUCLEIC  
ACID

**Title:** Mechanisms of Motility and Chemotaxis in Bacteria

**Institution:** CALIFORNIA INSTITUTE OF TECHNOLOGY PASADENA, CA

**Project Period:** 1982/08/01-2007/06/30

All living cells respond to their environment through sensory systems that detect specific changes in the chemistry or in the physical parameters of the environment and transduce these changes into intracellular second messages that change the metabolism and function of the cell. Bacterial chemotaxis is one of the best understood signal transduction systems and it has many characteristics in common with a variety of other signaling systems in microorganisms. Our long term goal is to be able to trace the information transduction process that underlies chemotaxis from ligand binding events through a series of stabilized protein conformational changes, to the mechanisms that lead to amplification and that explain the dynamic range of response of the system and all of the other physiological characteristics that this system displays. We have found that there are specific conserved modules that are expressed as domains of the proteins that interact to generate the chemotaxis signaling circuit. Homologs of these domains are distributed by evolution in a variety of different signaling systems where they play distinct and diverse roles in information processing. One of our goals is to understand exactly how these modules function. In the past, we have made great progress in determining the components of the chemotaxis system, understanding the physiology and behavior of the system and more recently in determining the atomic structure of the proteins involved in chemotaxis. It has become clear that there is a signaling complex that plays a critical role in information processing. Portions of this signaling complex can be reconstituted in vitro and we will study the chemotaxis proteins and genes from the thermophilic bacterium *Thermotoga maritima*. This system has many interesting characteristics that allow its manipulation in vitro. We have crystal structures for many of the components of the system that are required to generate the signaling complex. We will use a variety of biochemical, molecular biological, and biophysical tools to understand the mechanisms involved in signal transmission, signal generation, protein-protein interaction and information integration by these signaling complexes. This information obtained in vitro in the *Thermotoga* system will allow us to build specific test molecules that can be used in the *E. coli* system to correlate the atomic and molecular changes that occur in the signaling complex with the chemotaxis behavior of the organism. These insights will provide an understanding of the parameters that are critical to the function and evolution of microbial signaling systems and that control their versatility and ubiquity. Finally, comparable histidine kinases and signaling complexes are not present in mammalian systems. On the other hand, in many microorganisms and plants, these systems are essential for growth or pathogenesis. Thus, the histidine kinase represents an excellent target for the development of therapeutic agents, e.g. antibiotics, fungicides and herbicides. We will continue to explore this possibility.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials



**Grant:** 2R01AI019570-18  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** HATCH, THOMAS P PHD VET  
MEDICINE:MICROBIOLOGY  
**Title:** HOST FREE ACTIVITIES OF CHLAMYDIAE  
**Institution:** UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN  
CTR  
**Project Period:** 1983/09/01-2007/02/28

DESCRIPTION (provided by the applicant): The goal of my laboratory is to determine how gene expression is regulated in chlamydiae with the long term objective of understanding how chlamydiae cause latent, persistent, and chronic infections. The field of chlamydial research is severely handicapped by the lack of tools to genetically manipulate chlamydiae. However, the development of host-free (intact chlamydiae isolated from host cells) and in vitro transcription methodologies and the successful DNA sequencing of several chlamydial genomes offers new opportunities to identify regulatory DNA elements and transcriptional regulators that bind to these elements. Host-free and in vitro strategies will be used to accomplish four specific aims. 1. Determine the role of alternative sigma factors in gene expression in *Chlamydia trachomatis*. The temporal expression and the interaction of the sigma factors and their regulators will be characterized, and the promoters regulated by the sigma factors will be identified. 2. Determine the role of integration host factor (IHF) and histones in late gene expression. The genomic DNA binding sites of IHF and the chlamydial histone proteins will be determined, and the effect of histones and IHF on CRP-operon expression in vitro will be examined. 3. Characterize post translational modifications of chlamydial histone proteins. Transmethylation of histones by the chlamydial SET protein will be assayed in vitro with recombinant proteins, and the ability of modified histones to bind to chlamydial DNA will be determined. 4. Determine the pattern of gene expression during the developmental cycle, making use of host-free probes. A microarray consisting of all 894 chromosome and the 8 plasmid ORFs of *C. trachomatis* D will be probed directly with RNA synthesized by host-free chlamydiae at different times post infection. Emphasis will be placed on transcription at very early times post infection (pi).

**Grant:** 2R01AI019641-18A2  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** MURPHY, TIMOTHY F  
**Title:** Development of a vaccine for nontypeable H influenzae  
**Institution:** STATE UNIVERSITY OF NEW YORK AT AMHERST, NY  
BUFFALO  
**Project Period:** 1983/09/30-2007/02/28

DESCRIPTION: (Provided by Applicant) Nontypeable Haemophilus influenzae (NTHI) is an important cause of otitis media in children and lower respiratory tract infection in adults with chronic obstructive pulmonary disease (COPD). A vaccine to prevent otitis media in children and lower respiratory tract infections in adults with COPD would reduce mortality (in case of COPD) and morbidity, improve quality of life and reduce health care costs. The development of such a vaccine is one of the goals of the work proposed. The antigenic structure of two important outer membrane proteins of NTHI (P6 and P2) will be elucidated and their potential as vaccine antigens will be assessed. P6 has several characteristics suggesting that it will be an effective vaccine antigen. In Specific Aim 1, the structure of P6 as it exists in the outer membrane will be elucidated and the human immune response following immunization with P6 will be characterized with emphasis on identifying protective epitopes recognized by human antibodies. P2 is the major protein in the outer membrane. Antibodies to conserved regions of P2 are highly bactericidal for multiple strains of NTHI, indicating that these conserved regions may form the basis of an effective vaccine. In Specific Aim 2, the sequence heterogeneity of two relatively conserved surface loops will be determined, antibodies to conserved loops will be developed and characterized, the stability of P2 in the human respiratory tract will be assessed, and broadly cross-reactive human bactericidal antibodies will be studied for binding to conserved loops of P2. The proposed work will advance the area of vaccine development for NTHI by elucidating the antigenic structure of two important surface proteins and rigorously assessing their potential as vaccine antigens with emphasis on elucidating protective immune responses in humans.

**Grant:** 2R01AI024533-13A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** CURTISS, ROY PHD  
MICROBIOLOGY:MICROBIO  
OGY-UNSPEC  
**Title:** Molecular Genetic Analysis of Salmonella Pathogenicity  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 1987/04/01-2007/05/31

Our long-term objective has been, and will continue to be, to better understand the mechanisms governing infection and disease by Salmonella when administered by the normal oral route of entry. We will study *S. typhimurium* infection of chicks to evaluate persistent intestinal colonization and mice as a model of typhoid fever in humans and will make extensive use of murine and human cells in culture. We will continue, in all our endeavors, to develop methods to identify and analyze mechanisms for regulated expression of genes that might contribute to pathogenicity. Specifically, we will: (1) evaluate expression of *S. typhimurium* genes at ambient temperatures in a simulated polluted water environment with the objective to identify genes enhancing survival and potentiating successful colonization of the warm-blooded animal host and, subsequently, to characterize their functions and means of regulation, (2) define roles of adhesins in targeting Salmonella to specific cell types and tissues in the murine host, in enabling long-term colonization of the intestine and cecum in chicks, and in contributing to surface colonization (biofilm formation) in the simulated polluted water medium at ambient temperatures, and (3) continue to define mechanisms for colonization of the GALT (Peyer's patches) by identification of expressed genes with subsequent generation of mutants for characterization and complementation and to establish the means of their regulation. In these studies, we will extensively employ newly developed molecular genetic tools, such as selective capture of transcribed sequences (SCOTS), an easy and efficient method to generate mutant strains with defined deletion mutations, and selective regimens to generate operon fusions in addition to more standard means of genetic and molecular genetic manipulation. Our studies will use a broad range of methods of microbial genetics, molecular biology, biochemistry, immunology, cell biology, microscopy and animal science. All experiments will be conducted under conditions that preclude infections of workers and inadvertent release of infectious microorganisms.

**Grant:** 2R01AI025096-17  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** TAYLOR, RONALD K PHD OTHER AREAS  
**Title:** Genetic Determinants of Virulence in *Vibrio cholerae*  
**Institution:** DARTMOUTH COLLEGE HANOVER, NH  
**Project Period:** 1987/07/01-2007/04/30

DESCRIPTION (provided by applicant): The long range goal of the proposed work is to define the molecular components and mechanisms mediating *Vibrio cholerae* colonization and virulence protein secretion to the point where there is sufficient knowledge to intelligently incorporate this information into improved cholera vaccine strategies and antimicrobial therapies designed to inhibit these events. Most of the proposal involves analysis of the molecular mechanisms by which toxin coregulated pilus (TCP) is formed and mediates intestinal colonization. Some steps in the process by which TCP and other type 4 pili are built are linked and/or related to the process of toxin and other virulence determinant secretion by type II secretion systems. Thus further understanding of the mechanisms of type 4 pilus biogenesis could lead to the characterization of potential antimicrobial targets involved in multiple virulence pathways. We will examine the aspects of pilus biogenesis in detail. These experiments are facilitated by the development during the previous grant period of an in-frame deletion mutation in each gene encoding a component of the biogenesis apparatus and by the development of antibodies directed against most of the components of the apparatus. A second aspect of pilus biogenesis and toxin secretion to be addressed will be to undertake a more complete analysis of the prepilin processing reaction mediated by type four prepilin peptidases (TFPPs). These studies will build on the identification of the TcpJ TFPP as representative of a novel class of polytopic aspartyl proteases during the previous grant period and will focus on TcpJ interactions with TcpA prepilin substrate. Finally, the proposal focuses on the characterization of the TcpF protein. During the previous grant period, TcpF was shown to be an abundant secreted colonization factor. Secretion of TcpF is unique in that it is mediated by the pilus biogenesis apparatus. At this time, TCP and the secreted TcpF protein are the only major factors known to be required for colonization by *V. cholerae*, with tcpA and tcpF mutants of all epidemic serogroups and biotypes showing a 5 log decrease in colonization ability. The feasibility of incorporating TcpF into defined experimental cholera subunit vaccine formulations currently being developed will also be examined in this proposal. Taken together, the results of these studies will further our ability to design rational vaccination and therapeutic intervention strategies for cholera and other bacterial infectious diseases.

**Grant:** 2R01AI026815-15

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

**Principal Investigator:** BARTHOLD, STEPHEN W DVM VET  
MEDICINE:VETERINARY  
MEDICINE-OTHER

**Title:** Mouse Model of Lyme Borreliosis

**Institution:** UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA

**Project Period:** 1988/07/01-2007/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): Despite significant advances in understanding the biology of *Borrelia burgdorferi*, the agent of Lyme disease, the pathogenesis of Lyme disease remains poorly understood. The overall objective of this project is to use a mouse model of Lyme disease to investigate spirochete population kinetics and differential expression of specific *Borrelia burgdorferi* genes in selected target tissues during the dissemination/disease evolution phase, disease resolution (immune) phase, and persistent phases of *B. burgdorferi* infection. During the past project period, gene products were defined that are associated with antibody-mediated protective, but not arthritis- or carditis-resolving immunity (DbpA), and gene products that are associated with arthritis-, but not carditis- resolving or protective immunity (Arp and P37-42). In the current project period, the search will continue for other arthritis- and carditis-resolving antigen targets, as well as to characterize antigens expressed during persistent infection. Highly sensitive assays (real time PCR) have been optimized for quantitative analysis of spirochete population kinetics and for examining prototype gene expression in tissues at different stages of infection. Having optimized these assays, Specific Aim 1 will continue the search for *B. burgdorferi* antigens associated with biologically relevant antibody responses by screening a *B. burgdorferi* genomic expression library with sera from infected mice. Recombinant proteins and antisera will be tested for biologic activity (protective, disease-resolving, and dissemination-preventing activity) in the model. Specific Aim 2 will examine prototype gene expression during different phases of infection, and examine the effects of immunity or immune tolerance to biologically relevant antigens on these events. Specific Aim 3 will investigate mechanisms of persistent infection and disease quiescence by examining expression of prototype genes during this phase of infection, and define antigens that are involved in maintaining the host-agent equilibrium.

**Grant:** 2R01AI027243-14  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** BOOM, W HENRY  
**Title:** Heterogeneity of T-cells in M. tuberculosis Infection  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 1989/02/01-2007/03/31

DESCRIPTION (provided by the applicant): Mycobacterium tuberculosis is readily transmitted from person to person. In the majority of healthy persons acquired immunity, consisting of T cells and macrophages, controls growth but does not eradicate M tuberculosis. Persistent organisms can result in clinical tuberculosis when host immunity fails. The interaction of T cells and macrophages is central to this protective immunity. Macrophages serve as antigen presenting cells for M tuberculosis specific T cell and as effector cell for controlling mycobacterial growth. The macrophage also is the primary target for M tuberculosis infection and site of mycobacterial persistence. Despite activation of CD4+, CD8+ and g/d T cells with potent effector functions against M tuberculosis, the organism survives and persists. Thus the outcome of the interaction between M tuberculosis and acquired immunity is determined by the balance between the host mononuclear phagocyte's ability to process and present M tuberculosis antigens to T cell subsets, and M. tuberculosis' ability to modulate and resist macrophage antigen processing function. The M tuberculosis phagosome becomes the critical macrophage organelle where this dynamic balance is expressed. Proposed studies build on experimental systems and new results obtained during the last cycle of support of AI-27243. The three specific aims are: 1. To determine the role of the M tuberculosis phagosome as site for antigen processing of antigens for MHC class I restricted CD4+ T cells, and to determine the role of cytokines (IFN-g, IL- 10, TGF-B) and mycobacterial constituents such as the 19 kDa lipoprotein to modulate antigen-processing for CD4+ T cells. 2. To determine the mechanism for alternate processing of M tuberculosis for MHC class I restricted CD8+ T cells, to determine if alternate antigen processing is modulated to the same degree and by the same factors as for MHC class II antigen processing and to determine roles of the 44 kDa Rv0341 and 71 kDa Rv3808c proteins as antigens for CD8+ T cells. 3. To determine the role of M tuberculosis phagosomes in Vdelta2+gamma delta T cell activation by phosphoantigens, to determine the sensitivity to inhibition by IL-10, TGF-B and 19 kDa lipoprotein of processing of M tuberculosis by mononuclear phagocytes for gamma/delta T cells; to determine mechanism of gamma/delta T cell mediated M tuberculosis growth inhibition.

**Grant:** 2R01AI028457-11  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** YOTHER, JANET L PHD  
**Title:** Genetics and Virulence of Pneumococcal capsular types  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL  
BIRMINGHAM  
**Project Period:** 1989/07/01-2007/02/28

DESCRIPTION (provided by applicant): The capsular polysaccharides of *Streptococcus pneumoniae* are essential virulence determinants for this organism. They serve as the basis for serologic classification, with ninety serotypes being distinguished to date, and they are the target for protective antibodies. In both human infections and animal models, virulence is related to the serotype of the capsule expressed. The mechanisms underlying the virulence differences between serotypes are not known but may involve both the polysaccharide structures and other factors in the genetic background in which they are expressed. Molecular genetic studies have identified a common organization among the different *S. pneumoniae* capsule loci. This organization permits the exchange of capsular serotypes during transformation, leading to new combinations of virulence factors and potentially impacting on the efficacy of polysaccharide-based vaccines. Knowledge of the genetic components of the loci has permitted pathways for polysaccharide synthesis to be demonstrated or proposed. Often, enzymes expected to be essential for capsule production are not encoded by genes in these loci, and the necessary products are obtained from cellular pools contributing to pathways for peptidoglycan and teichoic acid synthesis. Thus, capsule expression and basic cellular functions are intimately linked. Recent studies have demonstrated a requirement for capsule in colonization but have suggested that capsule production is reduced in this environment as compared to other host environments. Expression of other factors important for adherence and sustained colonization may be elevated during colonization and reduced at other times. Coordinate regulation of these virulence factors is thus anticipated and recent studies have identified a surface component potentially involved in adherence whose expression is altered in response to changes in capsule production. In the proposed studies, we will continue the genetic analysis of virulence and capsule expression. The specific aims are to: 1) determine the effect of specific alterations in capsule type and structure on virulence, 2) characterize the requirement for capsule in pneumococcal infections and its expression in vitro and in vivo, and 3) characterize regulatory networks and cellular pathways associated with capsule synthesis.

**Grant:** 2R01AI029735-12  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** RADOLF, JUSTIN D MD CLINICAL MEDICAL SCIENCES, OTHER  
**Title:** Membrane Proteins of *Borrelia burgdorferi*  
**Institution:** UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT  
MED/DNT  
**Project Period:** 1990/04/01-2006/11/30

DESCRIPTION (Adapted from the Applicant's Abstract): *Borrelia burgdorferi*, the Lyme disease spirochete, is maintained in nature via a complex enzootic cycle that typically involves wild rodents and Ixodes ticks. To sustain itself, the spirochete must not only adapt physiologically to two markedly different host milieus, but it must also express virulence determinants and evade immune-mediated clearance mechanisms during mammalian infection. A finely orchestrated expression of arthropod- and mammalian host-specific genes, a process termed 'differential gene expression', is now believed to be responsible for many of these physiological, ultrastructural, and virulence-associated adaptations. Over the past several years, we and a number of other researchers have generated a substantial body of evidence that the bacterium's complement of linear and supercoiled plasmids comprises the primary substrate for genes expressed preferentially in vivo; this notion, however, requires more stringent examination. Equally important, despite the availability of the complete genomic sequence for the B31-MI isolate, Lyme disease researchers have made only modest progress in delineating and functionally characterizing the spirochete's complex array of differentially expressed genes, and we know even less about the mechanisms which regulate and coordinate these genetic programs. The underlying hypothesis of our proposal is that plasmid-encoded, differentially expressed genes are essential to the Lyme disease spirochete's parasitic strategy within the mammalian host. To address this hypothesis and its corollaries, we will characterize the differentially expressed portion of the *B. burgdorferi* proteome using two-dimensional electrophoresis and peptide mass fingerprinting (Specific Aim One); identify differentially expressed *B. burgdorferi* genes using high-density DNA microarrays (Specific Aim Two); continue our molecular and evolutionary analysis of *B. burgdorferi* cp32 plasmids (Specific Aim Three); and use newly developed plasmid shuttle vectors to study differential gene expression by *B. burgdorferi* (Specific Aim Four).



**Grant:** 2R01AI029904-12A1  
**Program Director:** LAMBROS, CHRIS  
**Principal Investigator:** ROSOWSKY, ANDRE  
**Title:** Lipophilic antifolates and AIDS opportunistic infections  
**Institution:** DANA-FARBER CANCER INSTITUTE BOSTON, MA  
**Project Period:** 1990/06/01-2006/02/28

DESCRIPTION (Provided by the applicant): The overall goal of this continuation project is the discovery of new drugs against *Pneumocystis carinii* (Pc), *Toxoplasma gondii* (Tg), *Mycobacterium avium* (My), and *Cryptosporidium parvum* (Cp), four opportunistic pathogens known to cause morbidity and mortality in AIDS patients. More specifically, the project will focus on the design and synthesis of several classes of mono- and dicyclic diamino-pyrimidine derivatives that we hope will combine the high potency of trimetrexate (TMQ) and piritrexim (PTX) with the species selectivity of trimethoprim (TMP) and pyrimethamine (PM) against Pc, Tg, or Cp dihydrofolate reductase (DHFR) versus rodent or human DHFR. The lack of binding selectivity of TMQ and PTX requires that they be used with leucovorin (LV) to prevent hematologic toxicity, whereas the relatively low efficacy of TMP and PM as single agents requires them to be used with sulfonamides and other drugs that often cause intolerable side effects. Thus, new DHFR inhibitors that are both potent and selective would be highly desirable. Compounds to be studied include several diaminopyrimidine ring systems with a short CH<sub>2</sub> bridge to the aryl side chain. The rationale for a short bridge is that, if the active site is sterically less accommodating in Pc or other non-mammalian DHFR than in mammalian DHFR, optimal hydrophobic contact should occur when the part of the inhibitor entering the active site is relatively compact (i.e., more like TMP than TMQ). We will also test the hypothesis that an effective way to achieve selectivity is with 2,4-diamino-5-[(2-methoxy- and 3,4-dimethoxy-5-(C3-9)alkoxy)-benzyl] pyrimidines containing an acidic carboxyl or tetrazole group at the end of the O-alkyl side chain. A very promising example of this class is 2,4-diamino-5-[2-methoxy-5-(4-carboxybutyl)benzyl]pyrimidine (PY657), which was recently discovered by us to have excellent potency and selectivity against both Pc and Ma DHFR. Also proposed are second-generation analogs of another lead compound we discovered in this project, N-(2,4-diaminopteridin-6-yl)methyldibenz[b,f]azepine (PT653). PT653 is selectively potent against Tg and Ma DHFR, but its bioavailability is limited by low aqueous solubility. Analogs of PT653 with a COOH, NH<sub>2</sub>, or OH group on the tricyclic moiety, and prodrugs thereof, will be made with the goal of improving aqueous solubility without sacrificing potency or selectivity.

**Grant:** 2R01AI031139-13

**Program Director:** TAYLOR, CHRISTOPHER E.

**Principal Investigator:** DERETIC, VOJO P PHD MOLECULAR BIOLOG  
OTHER

**Title:** Pseudomonas in Cystic Fibrosis-Regulation of Mucoidy

**Institution:** UNIVERSITY OF NEW MEXICO ANN ARBOR, MI  
ALBUQUERQUE

**Project Period:** 1992/02/01-2007/07/31

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* is the major cause of chronic respiratory infections in cystic fibrosis (CF) leading to persistent inflammation, lung tissue damage and high morbidity and mortality in this most common inheritable disease in Caucasians. The initially invading strains of *P. aeruginosa* are nonmucoid, but concomitantly with the establishment of a chronic infection, mucoid mutants overproducing the exopolysaccharide alginate emerge. The chronic infection, and additional host and bacterial factors that are not fully understood, lead to increased inflammation and irreversible tissue damage. This laboratory has previously described the mechanism of conversion to mucoidy which occurs via muc mutations that lead to the activation of the alternative sigma factor AlgU, the *P. aeruginosa* ortholog of the bacterial extreme stress sigma factor sigma-E. While alginate overproduction plays a role in reducing pulmonary clearance, we hypothesize that additional factors, co-expressed with alginate upon activation of AlgU, may contribute to pathogenesis in CF, since sigma factors normally direct transcription of a large number of gene subsets. So far, we have identified 10 additional genes controlled by AlgU that are activated in muc mutants. Importantly, a significant portion of these genes encode lipoproteins. Since lipoproteins play a role in innate proinflammatory signaling, we additionally hypothesize that *P. aeruginosa* products co-expressed with mucoidy contribute to inflammation in CF via pattern recognition receptors. Here we propose to: 1.further identify *P. aeruginosa* genes whose expression is activated in mucoid cells using conventional methods and techniques of global expression profiling; 2.analyze *P. aeruginosa* proinflammatory products associated with conversion to mucoidy; 3. analyze the role of pattern recognition receptors and signaling pathways involved in innate host response to *P. aeruginosa* products; and 4. study proinflammatory signaling in CF in combination with altered responses in CF cells. The specific aims of this proposal are to: 1) Identify *P. aeruginosa* genes that are activated or otherwise affected during conversion to mucoidy. 2) Determine how products of mucoid *P. aeruginosa* contribute to inflammation in CF. 3) Examine how proinflammatory products of mucoid *P. aeruginosa* synergize with the basic defect in CF and its downstream physiological effects, leading to exacerbation of pulmonary disease. These studies are expected to improve our understanding of respiratory pathogenesis in CF, and lead to new treatments of presently incurable lung infections associated with this disease.

**Grant:** 2R01AI031940-12

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

**Principal Investigator:** HOLMES, RANDALL K MD  
MICROBIOLOGY:MICROBIO  
OGY-UNSPEC

**Title:** Genetic Analysis of Cholera Toxin Structure and Function

**Institution:** UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO  
AURORA

**Project Period:** 1992/02/01-2007/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): Approximately 1.5 billion cases of diarrhea cause 4 million deaths annually in children under 5 years old, and 5-7 million cases of cholera cause about 100,000 deaths. Cholera toxin (CT) from *Vibrio cholerae* causes the massive watery diarrhea of cholera. Enterotoxigenic *E. coli* (ETEC) cause up to 20 percent of diarrheal disease in developing countries, and produce heat-labile enterotoxins called LTI and LTII that are closely related to CT in structure and function. The best current vaccines against cholera provide only moderate protection for short periods of time and are not licensed in the United States, and there are no vaccines for human use against ETEC. CT and related enterotoxins are potent immunogens and mucosal adjuvants, and they are also used widely as tools to investigate the role of heterotrimeric G proteins in signal transduction, the role of gangliosides in endocytosis and vesicular trafficking, the mapping and/or ablation of neural pathways, and many other cell functions. We study the structure and function of CT and use LTI and LTII in comparative studies to explore the molecular basis for functional differences between them. Our long term goals are to elucidate the molecular basis for biological activities of CT and related enterotoxins, and to use that knowledge to design novel structure-based vaccines and therapeutics to prevent or treat enterotoxigenic diarrheas. CT, LTI or LTII are also being studied widely as vaccine components, adjuvants or immunomodulators to prevent or treat diseases unrelated to enterotoxigenic diarrheas. Important issues concerning structure and function of CT that are not yet understood include identifying and characterizing: conformational changes that activate the catalytic capacity of CT-A1 after nicking and reduction of CT holotoxin; motifs on CT-A1 that determine its interactions with G $\alpha$ /G $\beta$ /G $\gamma$  as a substrate for ADP ribosylation and with ADP-ribosylation factors (ARFs) as stimulators of catalytic activity; features of CT-A and CT-B that enable them to assemble spontaneously into CT holotoxin; mechanisms by which binding of enterotoxins to plasma membrane receptors determines their trafficking within target cells; and pathway(s) by which CT-A1 is translocated from the ER to the cytoplasm to reach its intracellular target and cause toxicity. During the next project period we will use a wide variety of novel methods from microbiology, genetics, biochemistry, cell biology and structural biology to investigate these important current issues concerning the structure and function of cholera toxin.

**Grant:** 2R01AI032074-11  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** DONNENBERG, MICHAEL S MD  
**Title:** The eae Gene Cluster of Enteropathogenic E.coli  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 1992/02/01-2007/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): Enteropathogenic E. coli (EPEC) is an important cause of serious diarrhea in infants in developing countries and a model organism for studies of Enterohemorrhagic E. coli (EHEC) infections. These pathogens are able to attach intimately to host cells and induce dramatic changes in cytoskeletal organization in a process known as attaching and effacing that is dependent on the Locus of Enterocyte Effacement (LEE) pathogenicity island. Ongoing studies of the genes from one end of the LEE have provided important insights into the pathogenesis of EPEC and EHEC infection. Experiments designed to accomplish four specific aims will further these studies. The first aim is to identify the target(s) of and determine the mechanism of action of EspF. Two functions have been identified for EspF. EspF mediates host cell apoptosis and is required for disruption of intestinal barrier function. The mechanism of action of EspF will be revealed by identifying its host cell target(s). These studies will also determine whether these two functions are the result of a single or separate EspF activities. The second aim is to test the hypothesis that EspB has a role in attaching and effacing distinct from its role as a component of the translocation apparatus. EspB is required for translocation of EPEC effector molecules into host cells, but EspB is itself translocated to the host cell cytoplasm and EspB causes dramatic changes in epithelial morphology when transfected and expressed in host cells. Mutants with linker-insertion and point mutations in espB will be screened to determine whether EspB proteins that retain translocation activity but lack attaching and effacing activity can be identified. These studies will also yield a detailed analysis of EspB structure-function relationships. The third specific aim is to test the hypothesis that the product of orf23 is a component of the translocation apparatus and the fourth aim is to test the hypothesis that the product of orf27 is a chaperone for an EPEC effector protein that is not required for attaching and effacing activity. These hypotheses are firmly based on data developed during the current funding period and will lead to new insights into the structure of the translocation apparatus and into functions of the LEE other than attaching and effacing. The experiments described in this proposal are essential for improving our understanding of the pathogenesis of EPEC and EHEC infections.

**Grant:** 2R01AI032223-09  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** WEIS, JANIS J  
**Title:** Borrelia Burgdorferi Mitogen in Development of Arthritis  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 1993/04/01-2006/12/31

DESCRIPTION (provided by the applicant): Lyme disease is caused by the tick borne spirochete *Borrelia burgdorferi*. The bacteria possess outer surface lipoproteins possessing a tripalmitoyl-S-glycerylcysteine (Pam3Cys) modification to the amino terminal cysteine with potent pro-inflammatory potential. The signaling receptor for *Borrelia* lipoproteins has been identified as toll-like receptor 2, TLR2, a member of a family of molecules involved in innate responses to microorganisms. Members of this family, including the IL-1 receptor, signal through a well characterized pathway of signaling molecules, resulting in NF-kB translocation to the nucleus and transcriptional activation of numerous pro-inflammatory genes. The consequences of direct activation of this extensive inflammatory pathway during infection has profound implications for our understanding of disease process and host defense to this organisms. This application proposes to assess the involvement of TLR2 mediated signaling by lipoproteins in the host-pathogen interactions of Lyme disease. In Aim 1, the involvement of TLR2 in *B. burgdorferi* induced arthritis will be determined by infecting mice possessing a null allele for *tlr2*. Arthritis severity and composition of inflammatory infiltrate at various time points following infection will be assessed. Parameters of host defense will also be determined in these mice, by assessing levels of spirochete DNA in tissues at different time points following infection, and assessing the kinetics of appearance of *Borrelia* specific antibodies of various isotypes. In the second Aim, the contribution of TLR2-independent interactions to the host response to *B. burgdorferi* infection will be assessed. The bacterial component responsible for lipoprotein-independent responses will be characterized and it will be determined if the response requires the TLR/IL-1R signaling pathway. In Aim 3, antibody reagents will be developed to mouse TLR2, and used to identify responsive cells in ankle and heart tissues. The direct involvement of TLR2-lipoproteins in pathological development will be studied by determining the extent of pathology attributable to TLR2 bearing cells in inflamed tissues. In the fourth Aim, the structural requirements for TLR2 interaction with bacterial lipoproteins will be studied, using mutant constructs that display altered responsiveness. Demonstration of direct interaction between lipoproteins and TLR2 will be sought, as will the involvement of partners in cellular responses to the lipoproteins. All experiments are focused on characterization of the host-pathogen interactions involved in pathological consequences of Lyme disease.

**Grant:** 2R01AI032596-09A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** SCHREIBER, JOHN R  
**Title:** IgG subclass and function of anti-polysaccharide abs  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 1993/01/01-2006/05/31

DESCRIPTION (provided by applicant): Anti-polysaccharide (PS) antibodies (Ab) are critical to host defense against encapsulated bacteria. The function of anti-PS Ab includes complement fixation and opsonization of bacteria for killing by phagocytes, and modulation of cytokine producing cells via binding to Fcγ receptors (FcγR). IgG anti-PS Ab response has delayed ontogeny and isotype restriction (IgG3 in mice IgG2 in man), and conjugation of PS to proteins induces class switching to IgG1. Determining the effect of isotype restriction and IgG subclass on function of anti-PS Ab is crucial to better understand immunity to PS-encapsulated bacteria, the pathogenesis of human IgG subclass deficiency and for improved serological correlates of immunity with PS and PS conjugate vaccines. In this proposal, we will continue our studies of the role of IgG subclass in anti-PS Ab effector function, determine the role of constant region genes and the dominant IgG subclass in immunity to encapsulated bacteria and in class switching that occurs when PS are conjugated to proteins, and investigate the effect of IgG subclass on FcγR mediated regulation of cytokine production. We will utilize V region-identical human monoclonal Abs of all four IgG subclasses against *P. aeruginosa* LPS O-side chain and *S. pneumoniae* (Pn) capsular PS made in a new transgenic mouse reconstituted with human Ig genes, to determine the mechanism of functional differences in Ab protective efficacy. Next, to determine the in vivo relevance of the dominant IgG subclass made to PS, we will use the new BALB/c IgG3 knockout mouse to determine the importance of anti-PS Ab subclass in the host response to PS, PS-protein conjugates and to infection with encapsulated bacteria. We have found that the absence of IgG3 renders these animals more susceptible to fatal infection with Pn but that induction of anti-PS IgG1 can correct this defect. Finally, we will investigate the FcR mediated regulatory role of IgG subclass in cytokine production by macrophages, and we hypothesize that there are differences in the ability of IgG subclass to modulate cytokine production based on differential binding to FcγR. These studies will allow more rational strategies of active and passive immunization against bacteria, improved understanding of IgG subclass deficiency, and new information about which IgG subclass has the best FcγR-mediated anti-inflammatory properties.

**Grant:** 2R01AI033386-06A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** HAMOOD, ABDUL N PHD  
**Title:** Analysis of Toxin A synthesis in *P. aeruginosa*  
**Institution:** TEXAS TECH UNIVERSITY HEALTH SCIS LUBBOCK, TX  
CENTER  
**Project Period:** 1996/07/15-2007/02/28

DESCRIPTION (provided by applicant): Exotoxin A and the siderophores (pyochelin and pyoverdine) are produced in *P. aeruginosa* by a complicated process that involves several genes. The long-term goal of this proposal is to define the mechanism that regulates the synthesis of exotoxin A and pyoverdine in *P. aeruginosa* and to identify the different factors that participate in this regulation. We have identified a gene, *ptxR*, which positively regulates the transcription of *toxA*, the *toxA* regulator *regA*, and the genes that code for some components of the pyoverdine system. Available evidence suggests that the regulation of these genes by *ptxR* is indirect (through an independent gene). In addition, the regulation of *ptxR* expression itself appears to involve several regulatory proteins, both identified and as yet unidentified. One identified protein, the alternative sigma factor PvdS, also regulates *toxA* and the pyoverdine genes. The unidentified proteins bind specifically to the *ptxR* upstream region. The specific aims of this proposal are: 1) to determine the mechanism through which *toxA* and the pyoverdine genes are regulated by *ptxR*. This includes identification of the *ptxR* target sequence and the gene that carries it and characterization of the genes that are regulated (directly or indirectly) by *ptxR*; 2) to examine the regulation of *ptxR* expression in *P. aeruginosa*. This includes extensive *in vivo* and *in vitro* transcriptional analysis of the *ptxR* upstream region and examining *ptxR* expression in different specific *P. aeruginosa* mutants; and 3) to isolate and characterize the putative regulatory proteins and to identify the nucleotide sequences to which they bind. These aims will be accomplished using protein purification experiments, simple and modified DNA/protein binding experiments, functional genomics, 2-D gel analysis, *in vivo* and *in vitro* transcription, and different recombinant DNA techniques.

**Grant:** 2R01AI033696-11  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** BLANCHARD, JOHN S  
**Title:** Mechanism Based Inhibition of M Tuberculosis Enzymes  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 1992/09/30-2007/08/31

DESCRIPTION (provided by applicant): Bacterial resistance to antibiotics is a clinically significant problem that threatens current paradigms of antibacterial chemotherapy. This is particularly true for the treatment of tuberculosis, where singly- and multiply-drug resistant clinical strains of *Mycobacterium tuberculosis* have been identified with increasing frequency. Very few compounds are selective antimycobacterial agents, and mycobacteria are intrinsically resistant to many antibiotics either because of the constitutive expression of degrading enzymes (e.g., Beta-lactamases) or the inability of antibiotics to penetrate the uniquely hydrophobic outer cell wall. The principal investigator and his group have previously focused their attention on the DAP/L-lysine biosynthetic pathway, and will use similar approaches to explore the three-dimensional structures and chemical mechanisms of *M. tuberculosis* enzymes involved in the biosynthesis of pantothenate, a vitamin in mammals, and an essential pathway in *M. tuberculosis*. Specifically, they will clone, express and purify the *M. tuberculosis* *ilvGM*, *ilvC* and *ilvD* gene products involved in branched chain and pantothenate biosynthesis, and the *panB* gene product involved in pantothenate biosynthesis. They will clone, express and purify the *M. tuberculosis* *dxs*- and *dxr*-encoded deoxyxylulose-5-phosphate synthase and isomeroreductase for mechanistic comparison to the *ilvC*- and *ilvGM*-encoded enzymes. They will also determine the structures and chemical mechanisms of aminoglycoside N-acetyltransferases, enzymes that are primarily responsible for clinical resistance to aminoglycosides in both Gram-negative and Gram-positive bacterial pathogens. These enzymes catalyze a rich variety of chemistries, and plausible chemical mechanisms suggest that mechanism-based, and bi- and tri-substrate analogue inhibitors might be found. They will continue to use an integrated approach involving kinetic and chemical mechanism studies, three-dimensional structural studies, and site-directed mutagenesis to identify active site residues important for substrate and inhibitor recognition, and fulfill their long term goal of characterizing unique bacterial multi-step biosynthetic pathways that are absent in humans.



**Grant:** 2R01AI033706-11  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** MCNEIL, MICHAEL R PHD  
**Title:** Cell Wall Biogenesis-Target for new Anti-TB drugs  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 1992/09/30-2007/08/31

DESCRIPTION (Provided by applicant): In this competing continuation, mycobacterial cell wall metabolism is targeted as a route to new tuberculosis drugs. In one approach, essential cell wall biosynthetic enzymes are targeted. It is proposed to prepare *M. tuberculosis* luciferase reporter strains that respond specifically to inhibitors of the following essential enzymes: rhamnosyl transferase (WbbL), galactofuranosyl transferase (GlfT), dTDP-Rha formation enzymes (RmIB-D), and the UDP-Galf formation enzyme (Glf). In addition, direct enzyme assays amenable to screening for inhibitors will be developed for WbbL and GlfT (such assays are already in place for RmIB-D and Glf). It is further proposed to identify the genes encoding the three enzymes that form the arabinosyl donor, decaprenylphosphoryl-D-arabinose, show that their corresponding enzymes are essential, and prepare *M. tuberculosis* luciferase reporter strains and enzyme assays for use in finding inhibitors of them. In the second approach it is hypothesized that compounds that activate or deregulate the cell wall degrading arabinases and peptidoglycan hydrolyases can act more quickly than inhibition based drugs and thereby shorten tuberculosis therapy. Thus newly discovered *M. tuberculosis* endogenous arabinanases (of unknown function) that cleave cell wall arabinan will be purified and their genes identified. The effect of overexpressing these enzymes in *M. tuberculosis* will be tested; it is hoped the effect will be lethal. Reporter strains of *M. tuberculosis* to detect small molecules that up regulate the expression of the arabinanases will be prepared. The presence of a cell wall metabolic enzyme complex containing these arabinases (which can potentially be disrupted for therapy) will be searched for using a novel cross-linking reagent, cyanogen complexes of peptidoglycan synthetic and degradative enzymes, after covalent crosslinking with cyanogen, will be isolated. The identity of all proteins present in the complex will be determined by LC/MS and how individual proteins interact in the complex will be determined for the long-term goal of disrupting the complex to release the peptidoglycan cleaving enzymes. We will test *M. tuberculosis* for increased arabinase activity after exposure to novel highly active ethambutol like compounds (the presence of endogenous arabinase was first recognized during ethambutol treatment). Finally, large numbers of novel Betalactams will be tested for their action against *M. tuberculosis* with and without ethambutol like compounds anticipating that some will strongly stimulate cell wall degradation and bacterial death.

**Grant:** 2R01AI034276-08A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** SOUTHWICK, FREDERICK S  
**Title:** LISTERIA AND SHIGELLA USE HOST CELL ACTIN  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 1993/07/01-2007/05/31

DESCRIPTION (provided by applicant): The gram-positive bacillus *Listeria monocytogenes* predominantly infects immunocompromised patients, causing bacteremia and meningitis while the gram-negative bacillus *Shigella flexneri* infects normal hosts causing severe diarrhea and dehydration. The pathogenesis of Listeriosis and Shigellosis absolutely requires these intracellular bacteria to usurp the host cell's contractile system. *Listeria* and *Shigella* induce host cell actin to assemble into rocket tails that rapidly propel the bacteria through the cytoplasm, allowing their cell-to-cell spread and avoidance of the humoral immune system. Actin assembly occurs in a discrete polymerization zone directly behind the motile bacteria. This region blocks the host cell actin-regulatory proteins, gelsolin, CapZ and CapG, that normally cap the fast growing ends of actin filaments. This blocking activity allows actin filaments to rapidly assemble in this discrete zone. Two of these proteins, gelsolin and CapG, require micromolar calcium to function. We will: Aim I - Elucidate how *Listeria* blocks barbed end-capping proteins in the polymerization zone. Pyrenyl actin and right angle light scattering will be used to examine how profilin combined PIP2 and VASP or N-WASP effects actin filament capping by CapG, CapZ and gelsolin. Capping inhibition by *Listeria* will be investigated in brain cell free extracts before and after depletion of profilin and VASP. Localization of PIP2 (well known to block capping activity) in *Listeria* and *Shigella* infected cells will be studied using a GFP labeled probe. The effects of blocking PIP2 production using the PI kinase inhibitors Wortmannin and quercetin, infecting cells with *Listeria* ActA mutants lacking PIP2 binding sites, and ActA mutants lacking VASP binding sites will be examined. Aim II - Study the Calcium-Dependence of *Listeria* and *Shigella* actin-based motility. Calcium is a critical signal for turning on and off actin regulatory proteins, and we have found that the chelator BAPTAM blocks *Shigella* actin-based motility and slows the disassembly of *Listeria* rocket tails. The Ca<sup>2+</sup>-sensitivity of N-WASP and vinculin, cell proteins unique to *Shigella*-induced actin assembly, as well as gelsolin will be studied. These investigations should clarify key regulatory pathways required for *Listeria*- and *Shigella*-induced actin assembly and may identify new therapeutic targets for treating Listeriosis and Shigellosis.

**Grant:** 2R01AI036344-06A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** HANSEN, ERIC J PHD  
**Title:** Surface Proteins of Moraxella catarrhalis  
**Institution:** UNIVERSITY OF TEXAS SW MED DALLAS, TX  
CTR/DALLAS  
**Project Period:** 1996/07/01-2007/03/31

DESCRIPTION (provided by applicant): Moraxella (Branhamella) catarrhalis is now acknowledged to be an important cause of otitis media in infants and young children and can also cause lower respiratory tract infections in adults with chronic obstructive pulmonary disease. Little is known about the gene products that allow M. catarrhalis to colonize the nasopharynx and then cause disease in the respiratory tract. However, the ability to attach to human cells and to resist killing by normal human serum (i.e., serum resistance) are well-recognized bacterial virulence factors. We have identified two different proteins (UspA1 and UspA2) that are exposed on the surface of this pathogen and that perform distinct functions relevant to the ability of M. catarrhalis to colonize and survive in vivo. We already have established that UspA1 is an adhesin that binds human epithelial cells in vitro. We also have proven that UspA2 is directly involved in the expression of serum resistance by this organism. This research project involves investigation of the structure-function relationships inherent in these two proteins and also addresses two other topics that are relevant to the infectious process involving M. catarrhalis. In the first Specific Aim, we will identify the amino acid sequence(s) in the UspA1 protein that allows it to bind human epithelial cells. In the second Specific Aim, we will identify both the mechanism by which UspA2 confers serum resistance on M. catarrhalis and the amino acid sequence(s) in UspA2 responsible for this activity. Experiments designed to determine the level of UspA2 required for serum resistance and how UspA2 expression is regulated constitute the third Specific Aim. Finally, we will investigate biofilm formation by M. catarrhalis and identify gene products involved in this biologically relevant process in the fourth Specific Aim.

**Grant:** 2R01AI037230-07A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** MATHER, THOMAS N  
**Title:** Role of Tick Saliva in Lyme Disease and Vaccine Strategy  
**Institution:** UNIVERSITY OF RHODE ISLAND KINGSTON, RI  
**Project Period:** 1994/09/30-2007/03/31

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

**Grant:** 2R01AI037787-06A2  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** MARCONI, RICHARD T PHD  
**Title:** Immune Evasion Mechanisms in Lyme Disease  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 1996/06/15-2007/02/28

DESCRIPTION (provided by applicant): Lyme Disease (LD) continues to be a serious health problem in the USA. The chronic nature of the infection can lead to debilitating and serious clinical manifestations. While early diagnosis of LD is the key to successful treatment, accurate diagnosis continues to be a significant problem. In addition, while a vaccine has been developed and licensed there are concerns about the duration of protection it affords and about possible autoimmune responses (IR) in certain genetic backgrounds. These concerns highlight the need for a better understanding of the genetic and antigenic properties of this organism and the molecular mechanisms associated with chronic infection. Our studies on chronic infection and immune evasion by *B. burgdorferi* (Bb) have focused on a diverse group of plasmid-carried genes and operons that are 5'-flanked by a common upstream promoter-carrying sequence that we call the upstream homology box or UHB element. UHB-flanked genes are organized into 3 distinct gene families: the ospE family, the ospF family, and family 163 (a TIGR designation). The data described below demonstrate that the ospE gene family undergoes mutational and recombinational changes during infection. In addition, members of the ospF gene family are temporally expressed during infection. These different processes culminate in the presentation of new antigenic variants of OspE and OspF on the cell surface that can contribute to immune evasion. Little is known about the role of other UHB-flanked gene families in chronic infection and immune evasion. However, the polymorphic nature of these genes suggests that their organization has been influenced by recent molecular events that may include recombination and rearrangement. The major hypotheses of this proposal are that the OspE, OspF and family 163 proteins contribute to immune evasion in LD and play stage specific roles during infection. The analyses described within will also test the utility of these proteins in vaccine development.

**Grant:** 2R01AI037859-06  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** FLYNN, JOANNE L  
**Title:** Function of CD8 T Cells in Tuberculosis  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA  
PITTSBURGH  
**Project Period:** 1997/09/30-2007/03/31

DESCRIPTION (provided by the applicant): The immune response against Mycobacterium tuberculosis results in control, but not elimination, of infection. T cells are a crucial component of this response. This proposal extends our current work on examining the CD8 T cell subset and the effect of these cells in tuberculosis. It is clear that the naturally induced immune response is insufficient to resolve a M. tuberculosis infection. Understanding how the CD8 T cell response develops and is modulated over the course of infection may provide clues to augmenting this response to provide better control of infection. In this proposal, we will focus on following CD8 T cell responses to specific antigens, focusing on the function of the CD8 T cells at various times post-infection. Specifically, cytokine production and cytotoxic ability will be tested during acute, chronic, memory and reactivation states. Our hypothesis is that the CD8 T cell response and function wane during a chronic infection, and boosting this response would result in improved control of the infection. In addition, preliminary data indicates a role for CD4 T cells in maintaining CD8 CTL function, and the mechanisms responsible for this will be investigated. Our long term goal is to have a clear picture of the CD8 T cell response in tuberculosis, including antigen specificity, function, evolution, and maintenance. This information will impact directly on vaccine development, since it appears that stimulation of both CD4 and CD8 T cells will be necessary to provide adequate protection against tuberculosis. To this end, our specific aims are: Aim 1. To examine evolution in the antigen specific CD8 T cell responses during M. tuberculosis infection. Aim 2: To investigate the function of CD8 T cells in M. tuberculosis infection. Aim 3: To assess the development, maintenance and function of memory and recall CD8 T cell responses in tuberculosis. Aim 4: To determine the effects of CD4 T cells on CD8 T cell maintenance and function in tuberculosis. Animal models will be used in these studies, and we have adapted a variety of functional assays for CD8 T cells for use with lung cells. These complementary aims will provide a definitive picture of the CD8 T cell response in tuberculosis, and contribute to a greater understanding of the challenges facing vaccine development and design against this disease.

**Grant:** 2R01AI038178-06A2  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** ANDERSON, BURT E PHD OTHER AREAS  
**Title:** Bartonella henselae antigens of biological significance  
**Institution:** UNIVERSITY OF SOUTH FLORIDA TAMPA, FL  
**Project Period:** 1995/07/01-2006/03/31

DESCRIPTION (Provided by the applicant): The bacterium *Bartonella henselae* causes a variety of disease syndromes including severe systemic life-threatening infections in AIDS patients. The NIH Plan for HIV-Related Research (FY2002) lists objective 2F as "elucidate the pathogenic mechanism of HIV-related opportunistic infections in adults and children." Despite the recognition of *B. henselae* as an emerging pathogen, very little is known about the novel mechanisms by which this bacterium causes disease. This project targets the most unique aspect of *Bartonella* pathogenesis (angiogenesis), that is observed primarily in AIDS patients with bacillary angiomatosis (BA) and bacillary peliosis hepatis (BPH). The central hypothesis of this proposal is based on a new model of *B. henselae* -induced angiogenesis that we have advanced requiring a major role for surface proteins and structures of *B. henselae* in this process. The following specific aims are proposed to test the hypothesis: 1) determine the role each of the four major surface structures of *B. henselae* play in interaction with host endothelial cells, 2) examine the ability of *B. henselae* and each of the four major surface structures of *B. henselae* to induce production of vascular endothelial growth factor (VEGF), 3) determine if VEGF produced by interaction of *B. henselae* with other cell types is able to function in a paracrine manner to induce proliferation of human microvascula endothelial cells. In vitro cell culture systems will be utilized for both the induction of VEGF (macrophages, fibroblast! epithelial cells) as well as the proliferation of endothelial cells leading to angiogenesis. The definition of the pathogenic processes utilized by *B. henselae* will help us better understand why this bacterium causes a mild disease in healthy individuals and severe diseases including BA and BPH in AIDS patients.

**Grant:** 2R01AI038281-06A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** O'BRIEN, ALISON D PHD  
MICROBIOLOGY:MICROBIO  
OGY-UNSPEC  
**Title:** Rho-Modifying Cytotoxic Necrotizing Factor of E. coli  
**Institution:** HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD  
MIL/MED  
**Project Period:** 1995/09/30-2007/04/30

Cytotoxic necrotizing factor type 1 (CNF1) is a member of a family of bacterial toxins that target the Rho family of small GTP-binding proteins in mammalian cells. CNF1 deamidates a single glutamine residue in RhoA, Cdc42, and Rac1 but not in Ras. This deamidation results in the constitutive activation of these GTPases which can trigger actin stress fiber formation, multinucleation, or cell death, depending on the target cell and dose of toxin. CNF1 is frequently produced by *Escherichia coli* strains that cause urinary tract infections (UTIs), such as cystitis, prostatitis, and pyelonephritis. In support of this epidemiological connection, we recently showed that CNF1 not only induces apoptosis in 5637 human uroepithelial cells but also provides a growth advantage to uropathogenic *E. coli* (UPEC) in a mouse model of ascending UTI when compared to CNF1-negative isogenic mutants. Additionally, we found that CNF1 enhances the degree of inflammation and resulting tissue damage in bladders of infected mice and in prostates of rats challenged intraurethrally with CNF1-producing UPEC. Finally, we discovered that CNF1-producing UPEC survive better than CNF1-negative isogenic mutants in the presence of human polymorphonuclear leukocytes (PMNs). Taken together, these findings have led us to propose the following hypothesis. CNF1 enhances the pathogenicity of UPEC by: i.) promoting uroepithelial cell shedding; ii.) evoking a large influx of PMNs while providing toxin-producing *E. coli* protection against PMN-mediated killing, and; iii.) facilitating deeper invasion of the bladder or prostate by the infecting strain. The specific aims designed to test this theory are to: 1.) further define the role that CNF1 plays in the pathogenesis of UPEC-mediated cystitis in the mouse and prostatitis in the rat by analyzing the interaction of CNF1 or CNF1-expressing UPEC with PMNs from these animals and by defining the CNF1-mediated cytokine response that evokes PMN influx; 2) investigate the cellular and cytokine responses of a human bladder organoid to CNF1 or a CNF1-producing UPEC strain; 3.) identify the functional receptor for CNF1 by sequential biochemical and molecular approaches, and; 4.) continue to evaluate CNF1 structure and function by characterizing the CNF1 epitopes recognized by neutralizing monoclonal antibodies and by analyzing chimeric molecules comprised of portions of CNF1 and the related toxins CNF2, *Pasteurella multocida* toxin, and the *Bordetella dermonecrotic* toxin.



**Grant:** 2R01AI038424-05A2  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** MADOFF, LAWRENCE C MD  
**Title:** Group B Streptococcal Surface Proteins Immune Evasion  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 1997/03/01-2006/01/31

DESCRIPTION (provided by applicant): Group B Streptococcus (*Streptococcus agalactiae*) remains a major cause of death and serious illness in newborn babies and has emerged as an important pathogen in immunocompromised adults. Our work on this pathogen has focused on a group of immunologically important surface proteins, the prototype for which is the alpha C protein. These protective surface proteins are characterized by the presence of long tandem repeating subunits. Variation in the number of tandem repeats gives rise to antigenic variation and permits escape mutation. During the first funding period for this project, we established that deletions of tandem repeats within the alpha C protein gene give rise to changes in the antigenic structure of the protein, alters its immunogenicity, and affects its positioning on the bacterial surface. These changes underlie the ability of group B streptococci to escape from host immunity to this antigen. New data show a role for the alpha C and alpha-like proteins in binding and invasion of epithelial cells. In this renewal, we will examine the alpha C protein with respect to its adaptive advantage for group B streptococci. Specifically, we will examine mechanisms for regulation of expression of the proteins and study how the proteins mediate the interaction of streptococci with host epithelial cells. These studies will lead to advances in the understanding of group B streptococcal immunity, further the effort to employ these proteins in vaccines for the prevention of group B streptococcal infection, and enhance the understanding of pathogenesis at the most fundamental level.

**Grant:** 2R01AI039031-07A1  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** PAMER, ERIC G MD IMMUNOLOGY  
**Title:** Inflammation and T cell immunity to Listeria  
**Institution:** SLOAN-KETTERING INSTITUTE FOR NEW YORK, NY  
CANCER RES  
**Project Period:** 1996/07/01-2007/02/28

DESCRIPTION (provided by applicant): The murine immune response to Listeria monocytogenes infection is rapid, robust and highly effective at providing long term protective immunity. We have characterized the expansion of L. monocytogenes specific CD8 T cells during in vivo infection and find that the duration of T cell proliferation is not influenced by the duration or severity of infection. The experiments described in this grant application will test the hypothesis that CD8 T cells and the immune environment are programmed during the first 24 to 48 hours of infection by the innate inflammatory response, and that subsequent expansion and memory formation occurs independently of in vivo antigen presentation or infection induced inflammation. Our first aim is to test this hypothesis by transferring antigen specific T cells from infected mice into recipients that are uninfected, infected with antigen deficient strains of L. monocytogenes or infected for different durations with wild type bacteria. These studies will characterize the in vivo impact of inflammation and antigen presentation on antigen specific T lymphocytes expansion and memory formation. Our second aim is to characterize the CD8 T cell response to immunization with heat killed L. monocytogenes (HKLM). Our preliminary studies demonstrate that immunization with HKLM induces antigen specific CD8 T cell proliferation, but the duration and extent of proliferation is attenuated compared to that induced by live infection. We have designed experiments to determine the role of CD40 and IL-12 in the programming of CD8 T cell expansion. The third aim is to determine the role of innate inflammation induced by TLR signaling and by inflammatory chemokines on CD8 T cell expansion and memory formation. We will use mice deficient in TLR-2, TLR-4, MyD88, MIP-1a, CCR2 and CCR5 for these studies. Understanding the mechanisms that drive in vivo T cell expansion and memory information is important, since potent vaccines should be designed to elicit robust, long lasting T cell responses. Our studies will shed light on the important interactions between innate immunity and adaptive T cell responses.

**Grant:** 2R01AI039108-05  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** BAYER, ARNOLD S  
**Title:** Staphylocidal Mechanism of Platelet Microbicidal Protein  
**Institution:** HARBOR-UCLA RESEARCH & EDUC INST TORRANCE, CA  
**Project Period:** 1996/12/01-2006/11/30

DESCRIPTION (provided by the applicant): *Staphylococcus aureus* is a virulent pathogen which is associated with a broad-spectrum of clinical infections. Its ability to colonize host tissues, and to persist and proliferate within host tissues requires the organism to circumvent innate host defense mechanisms. We have discovered that mammalian platelets store and secrete a family of antimicrobial peptides at potential sites of endovascular damage and microbial colonization that serve to both growth inhibit and kill *S. aureus*. In the previous grant period, we have delineated that the principal antimicrobial peptide which is secreted from platelets (thrombin-induced platelet microbicidal protein-1 [tPMP-1]), interacts with *S. aureus* in vitro by initial attachment to the cytoplasmic membrane, after which a microbicidal cascade is triggered in strains intrinsically susceptible to this peptide. In contrast, those strains which were engineered to be resistant to tPMP-1 in vitro (e.g., by transposon mutagenesis) do so by changing the basic biology of their cytoplasmic membrane target for tPMP-1. In vitro susceptibility to tPMP-1 is mirrored by enhanced clearance of such strains in animal models of endovascular infection; in contrast, in vitro resistance to tPMP-1 is correlated with an augmented survival advantage in the same animal models. The overall purposes of this proposal are: i) to define the mechanisms by which tPMP-1 executes its microbicidal effects, particularly focusing on intracellular targeting and activation of stress response systems; and ii) to delineate the mechanisms, genetic pathways and membrane biochemical adaptations by which the organism is able to successfully respond to exposures to tPMP-1 for survival. For these purposes, we will utilize a series of well-characterized and isogenic strain pairs of *S. aureus* (including site-directed plasmid mutants, as well as mutants with plasmid reporter fusions) that will enable us to define both the mechanisms of microbicidal action of tPMP-1, as well as the homeostatic adaptive pathways used by the organism to survive tPMP-1 exposures. Moreover, we will employ proteomics approaches to divulge novel genes and metabolic pathways triggered by tPMP-1 as part of either its microbicidal cascade, or as part of the organism's adaptive strategies. These studies will provide a solid foundation for the future design of unique platelet peptide congeners which are better able to target *S. aureus* strains for killing, as well as to circumvent innate homeostatic mechanisms used by the organism for survival.

**Grant:** 2R01AI039482-06  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** TUOMANEN, ELAINE I  
**Title:** CONTROL OF AUTOLYSIS IN PNEUMOCOCCI  
**Institution:** ST. JUDE CHILDREN'S RESEARCH HOSPITAL MEMPHIS, TN  
**Project Period:** 1997/07/01-2007/06/30

DESCRIPTION (provided by applicant): Regulation of bacterial autolytic enzymes (cell wall hydrolases) is a highly sophisticated physiological task. Antibiotics such as penicillin induce bacteriolysis by interfering with the control of the endogenous autolytic enzymes, indicating the major chemotherapeutic relevance of autolysins. Although the binding of antibiotics to cell wall synthetic enzymes has been very well characterized, it is unknown how this event leads to deregulation of autolytic enzymes. It is this aspect of antibiotic activity, revealed as the tolerant phenotype, that is the focus of this proposal. Bacteria which stop growing in response to penicillin but fail to lyse and die are termed tolerant. This property, first described in pneumococcus, ensures bacterial survival and is the first step for most strains on the way to development of antibiotic resistance. During the first 5 years of this proposal, 5 genetic loci were identified which produced tolerance when mutated in pneumococcus. These are the first members identified in an autolytic cascade. Two of the loci defined a signal transduction apparatus triggering autolysis. This proposal seeks to build on these findings by characterizing in detail the mechanism of signal transduction which results in lysis. In particular, the structure and metabolism of the death peptide signal will be elucidated and a potential second peptide signal will be characterized. To identify more elements in the autolysis cascade, two approaches will be taken. Two additional tolerant mutants will be studied in detail. Second, elements in the VncR regulon affected by the VncR DNA binding protein will be sought. Finally, the significance of tolerance in the clinical setting will be defined by improving diagnostics for this trait and investigating the impact of tolerance on the course of infection in animal models. This will provide information important to the development of new potential antibacterial agents and perhaps suggest why bacteria in the clinical environment choose to regulate autolytic activity rather than dispense with suicidal autolysins in the face of antibiotic pressure.

**Grant:** 2R01AI040124-06  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** BAUMLER, ANDREAS J PHD  
**Title:** Analysis of Salmonella typhimurium Fimbrial Antigens  
**Institution:** TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX  
CTR  
**Project Period:** 1997/05/01-2007/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): Salmonella serotypes are the leading cause of food-borne infections with lethal outcome in the United States. The role of fimbrial adhesins in colonizing intestinal surfaces has not been intensively studied in Salmonella, but likely represents an important first step during infection. Our long-range goal is to understand the role of fimbrial adhesins in Salmonella pathogenesis. The objectives of this application are to determine the effect of fimbrial phase variation on bacterial populations (in vitro and in vivo) and to define the contribution of these phase variable antigens to virulence using the mouse typhoid and bovine enterocolitis models. Our central hypothesis is that phase variation is an immune evasion mechanism of a group of functionally related fimbrial antigens that act in concert during intestinal colonization by *S. typhimurium*. This hypothesis has been formulated based on strong preliminary data, which suggest that (i) fimbriae elicit an adaptive immune response that can be evaded by phase variation, (ii) the *S. typhimurium* genome contains a large number of fimbrial operons that are regulated by phase variation, and (iii) simultaneous inactivation of multiple fimbrial operons has a synergistic effect on virulence. The rationale for the proposed research is that a better understanding of factors involved in intestinal adherence will likely provide new insights into mechanisms of tissue tropism, host range and disease caused by *S. typhimurium* that are required for new and innovative approaches to prevention and treatment. We plan to test different aspects of our hypothesis by pursuing the following four specific aims: (1) Determine the consequences of fimbrial phase variation on the heterogeneity of a *S. typhimurium* culture; (2) Determine the serological response to fimbrial subunits; (3) Determine the effect of an immune response elicited by vaccination with fimbrial subunits on *S. typhimurium* virulence; (4) Determine the role of fimbrial operons during intestinal colonization. It is our expectation that our approach will establish that fimbrial adhesins are components of a complex virulence factor required for intestinal colonization. This outcome will be significant since it will establish a new paradigm in Salmonella pathogenesis. The research will be of additional significance since it will shed light on the role of adherence in the pathogenesis of enterocolitis, a disease syndrome that is common in the US but only poorly understood because most investigators rely on a typhoid fever model to study virulence mechanisms.

**Grant:** 2R01AI040247-05A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** STEPHENS, DAVID S  
**Title:** Regulation of Capsule Biosynthesis in *N. meningitidis*  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 1997/07/01-2007/02/28

DESCRIPTION (provided by applicant): Capsular polysaccharides of serogroups A, B, C, Y and W-135 are major virulence factors of the important human pathogen *Neisseria meningitidis*. Despite the essential role of capsule in meningococcal disease, the genetic basis for expression of meningococcal capsules is not fully defined. In this continuation application, we propose to determine the pathways regulating meningococcal capsular polysaccharide assembly and transport to the cell surface. In Specific Aim 1, we will define the genetic basis for formation of the meningococcal capsule polymer. New genes to be studied include the unique genes *sacA-D* of the serogroup A capsule biosynthesis locus, and two genes, *kpsF* and *pglB2*, located outside the capsule locus, that when mutated reduce meningococcal capsule expression in all serogroups and increase sensitivity to killing by human sera. Preliminary data indicate that *kpsF*- and *pglB2*-encoded proteins influence Kdo and undecaprenol phosphate production and that these intermediates are required for capsule polymer assembly. In Specific Aim 2, the genetic and biochemical basis for the novel lipid modifications (1,2 diacylglycerol C16:0 or C18:0) of meningococcal capsule polymers will be determined and the role of *lipA* and *lipB* in lipidation of the capsule polymer, capsule assembly, transport and function will be defined. In Specific Aim 3, the function of the *ctrA*, *ctrB*, *ctrC* and *ctrD* capsule transport operon genes and their encoded proteins will be determined. Very limited information is available regarding the mechanisms of meningococcal capsule translocation across the two cellular membranes and about the recognition and interaction between the transport apparatus and the assembled capsule polymers. The proposed studies have direct application to the development of new meningococcal vaccine strategies. *CtrA*, for example, is a surface-exposed protein, appears conserved, is essential for capsule expression and is a candidate for conjugate or other meningococcal vaccines that prevent disease due to all invasive serogroups. Antibiotic resistance is also increasing in meningococci. These studies also can identify novel pathways that interfere with meningococcal capsule expression, which, when blocked, would increase meningococcal susceptibility to killing by host defenses and decrease meningococcal transmission to new human hosts.

**Grant:** 2R01AI040488-06A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** ORME, IAN M  
**Title:** Defined Native Antigens and Immunity to tuberculosis  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 1996/03/01-2007/05/31

DESCRIPTION (provided by applicant): The purpose of this request for competing continuation funding is to continue our work on dissecting and defining the fundamental nature of the host immune response in the lung after exposure of mice to a realistic low dose aerosol infection with *Mycobacterium tuberculosis*. While our past work has contributed significantly to the definition of the acquired response, it is now clear that early resistance in the lung to infection is regulated by potentially redundant layers of very poorly understood innate immune mechanisms in addition to the better-characterized TH1 protective response. In the next funding period we propose a series of experiments to define these early mechanisms more clearly using a variety of approaches including flow cytometry, high speed fluorescence-activated cell sorting, histology, and immunohistochemistry. In our first Aim we will continue to try to identify sources of IFN-gamma that appear very early during the course of the infection, concentrating on the role of NK/NKT cell subsets and their potential restriction by Class-Ib MHC encoded molecules. In a second Aim, we propose to continue studies that are constructed to define the basic requirements needed to adequately and efficiently focus lymphocytes into the infected lungs, using a combination of flow cytometric analysis and sorting followed by adoptive transfer of tagged specific T cell subsets and subsequent tracking. Finally, we propose a fresh look at the memory T cell response in tuberculosis, specifically concentrating on parameters such as longevity, turnover, and potential cell surface marker reversion, primarily defined by flow cytometry. The proposed work will draw upon the broad expertise of various members of the *Mycobacteria* Research Laboratories at Colorado State University, as well as several eminent consultants and advisers.

**Grant:** 2R01AI040725-06A1  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** RYAN, EDWARD T  
**Title:** Mucosal Immunity to Antigens Expressed by V.cholerae  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 1996/12/01-2007/06/30

DESCRIPTION (provided by applicant): Clostridium difficile is the cause of significant morbidity, mortality and cost. Systemic and mucosal immunity to C. difficile toxin A has been associated with protection from clinical disease, amelioration of clinical symptoms, and prevention of relapse. The previous funding period of this grant resulted in several advances in the use of live, oral, attenuated Vibrio cholerae as a vector for immunizing against heterologous antigens. The long term goal of the next, proposed segment of funding is to develop and test in animals a V. cholerae-based vector vaccine that will stimulate systemic and mucosal humoral immunity against toxin A of C. difficile, and that is appropriate for ultimate human use. The current proposal has FOUR SPECIFIC AIMS to achieve this long term goal. These aims are: 1. To develop and analyze in vitro an anti-C. difficile V. cholerae-based vaccine vector. A glutamine auxotroph of V. cholerae vaccine strain CVD 103-HgR (a safe and immunogenic vaccine strain in North American human volunteer studies) will be engineered to express toxin A-HlyA (a fusion protein between a non-toxic 720 amino acid C. difficile toxin A fragment and the E. coli hemolysin A secretion signal) from plasmids of various copy numbers that complement the glutamine auxotrophy. The vaccine vector strains will also express HlyBD (the pore-forming proteins that mediate extracellular secretion of toxin A-HlyA), and an immunoadjuvant molecule, such as LT(R192G) (a non-toxic derivative of Escherichia coli heat-labile enterotoxin that retains immunoadjuvancy). Expression and cellular localization of toxin A-HlyA and LT(R192G) will be evaluated, as well as viability and stability of the vaccines in vitro. 2. The viability, stability, immunogenicity, and reactogenicity of the various oral vaccine constructs will then be evaluated in mice. 3. A combination oral priming and transcutaneous boosting immunization strategy (the latter with C. difficile toxin A toxoid with or without an immunoadjuvant) will be evaluated in mice for production of both mucosal and systemic immunity to toxin A. 4. The immunogenicity, reactogenicity, and protective efficacy of the most promising vaccine strategy will be assessed in rabbits, the latter will be measured using a challenge assay in which purified C. difficile toxin A is injected into ligated ileal loops of vaccinated and control animals.



**Grant:** 2R01AI041213-05A1  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** DUMLER, JOHN S MD MEDICINE  
**Title:** Virulence and Immunity of Granulocytic Ehrlichiae  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1996/08/01-2006/04/30

Human granulocytic ehrlichiosis (HGE) is an emerging tick-borne infection caused by *Anaplasma* (Ehrlichia) phagocytophila, an obligate intracellular bacteria that infects neutrophils. The infection is widespread and severity ranges from fatal to subclinical; the seroprevalence reaches 15 percent in some areas. We characterized antigenic variation in the major immunodominant surface protein (Msp) of the bacterium that likely results from differential transcription of one of at least 18 different gene copies. Moreover, we also recently discovered that pathogenicity in a murine model is due to induction of immunity, chiefly interferon-gamma, and is abrogated by interleukin-10, despite the presence of large numbers of bacteria. Because of these observations, we hypothesize that induction of pathologic lesions and disease depend upon immunity that varies with changing bacterial factors, possibly Msp. To test this hypothesis and to more completely understand pathogenetic mechanisms of granulocytic ehrlichiae, we will use a convenient mouse model and validate results in the horse model that mostly closely simulates human disease. Thus, we propose to: 1. Characterize the diversity and induce changes in expression of *A. phagocytophila* major surface protein genes in vitro and in vivo. Partial Msp cDNA transcripts from infected humans and animals will be sequenced and compared to identify evidence of ongoing gene transcriptional changes, and laboratory strains will be induced to alter the expressed Msp by specific-Msp antibodies, prolonged in vitro cultivation, or in vivo infection. 2. Demonstrate differences in pathology and immune responses after challenge with virulent (low passage) and reduced virulence (high passage) *A. phagocytophila*. Mice and horses infected with strains of ehrlichiae that differ in virulence will be assessed for T cell and cytokine phenotype and results will be correlated with clinical signs and pathology. 3. Demonstrate that innate immune responses to whole ehrlichiae and component Msps comprise part of the early proinflammatory response. Innate responses to native components of virulent and attenuated virulence ehrlichiae will be assessed for their ability to initiate inflammatory and immune reactions via toll- like receptors (TLRs), IL-12; IL-18, and NK cells in vitro and in vivo in knockout mice. Immunophenotype and cytokine profiles will be correlated with clinical signs or pathologic lesions in the infected animal models for both. 4. Characterize how the components of the adaptive immune response to whole ehrlichiae and component Msp influence the histopathology and clinical signs in GE. The role of specific immune cells (CD4, CD8, and NK) as well as specific effector mechanisms (nitric oxide, reactive oxygen intermediates, perforin, complement) that can lead to cell injury, histopathology, and clinical signs will be assessed by in vivo analysis of gene knockout mice, and in horses by corticosteroid- blockade of Th1 immunity. Immune cell and cytokine profiles will be analyzed and correlated with pathology and clinical signs. The demonstration in animal models of the major pathways by which immune and inflammatory reactions induced by ehrlichiae cause pathologic lesions and clinical signs would be a major advance. Since in vitro growth modifies virulence, defining a linkage between bacterial modifications and immunopathologic reactions would provide a more complete understanding of

the roles that antigenic and genetic variation play in the ehrlichioses or other infections. This information will provide the framework for rational design of new therapies aimed at modulating immune mechanisms and design of preventative measures aimed at immunization.

**Grant:** 2R01AI041513-05A1  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** SULLAM, PAUL M MD  
**Title:** Role of Streptococcal-Platelet Binding in Endocarditis  
**Institution:** NORTHERN CALIFORNIA INSTITUTE RES & SAN FRANCISCO, CA  
EDUC  
**Project Period:** 1997/07/01-2007/06/30

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. The goal of this project is to characterize further the molecular basis for direct platelet binding by *Streptococcus gordonii*, and the role of binding in the pathogenesis of endocarditis. We have identified two loci of *S. gordonii* strain M99 that mediate the direct binding of this organism to human platelets in vitro. The first locus (*gspA*) encodes a 117 kDa cell wall-associated protein that may function as an adhesin. The second locus appears to be an operon (*gspB-secY2A2*) encoding a 286 kDa cell wall anchored protein (GspB) that is also a likely platelet binding protein. In addition, this operon contains genes (*secA2* and *secY2*) that are required for the export of GspB. We now seek to further examine the role of these genes and their products in mediating binding to platelets. Our first goal is to characterize more extensively these loci by confirming that expression of *gspA* or *gspB* is linked to platelet binding. This work will include complementation studies of *gspA* and *gspB* mutants, expression of GspA or GspB in *Lactococcus lactis* and studying its effects on platelet binding by that organism, and additional mapping of the *gspB-secY2A2* operon. We will then purify GspA and GspB, and examine the binding properties of each protein with human platelets in vitro, thereby determining if binding resembles a receptor-ligand interaction. Purified GspA and GspB will also be used to identify their respective platelet binding sites. To assess the role of these adhesins in the pathogenesis of endocarditis, we will compare the virulence of M99 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:** 2R01AI041558-06  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** SKORUPSKI, KAREN A PHD  
**Title:** New Mechanisms for Regulating Virulence Gene Expression  
**Institution:** DARTMOUTH COLLEGE HANOVER, NH  
**Project Period:** 1997/07/01-2007/07/31

DESCRIPTION (provided by the applicant): Elucidating the mechanisms by which pathogenic bacteria regulate virulence gene expression in response to environmental stimuli is considerably important since this knowledge is central to understanding the molecular events that lead to the establishment of bacterial infections. *Vibrio cholerae* is the causative agent of epidemic cholera and possesses two distinct pathogenicity islands required for disease: the *Vibrio* pathogenicity island (VPI), which encodes an essential colonization factor, toxin-coregulated pilus (TCP), and the CTX element that carries the genes for cholera toxin (CT). We have recently identified two new activators, AphA and AphB, which function at what appears to be the initial regulatory step in the virulence transcriptional cascade. AphA and AphB function synergistically to activate the expression of the membrane bound transcriptional activators, TcpP and TcpH, which are encoded on the VPI. AphA appears to be a novel activator with no known homologs and AphB is a member of the LysR family. Interestingly, these two proteins are not encoded on either the VPI or CTX islands but are located in regions of the chromosome not previously associated with pathogenesis. Since activation of tcpPH expression by AphA and AphB occurs only under certain environmental conditions, these proteins may play a role in normal cellular physiology and function to couple physiological responses to virulence gene expression. The importance of AphA and AphB in pathogenesis is reflected by the dramatic attenuation in the infant mouse cholera model of mutants defective in either one of these genes. Furthermore, activation of tcpPH by AphA and AphB is responsible for the differential regulation of virulence genes between the two disease causing biotypes, classical and El Tor. The proposed research will allow us to gain a better understanding of the roles of AphA and AphB in pathogenesis. We propose: (1) to investigate the mechanism by which AphA and AphB differentially activate the expression of the classical and El Tor tcpPH promoters; (2) to determine the mechanism by which cAMP-CRP antagonizes AphA and AphB to negatively regulate tcpPH expression; (3) to elucidate the molecular mechanisms by which environmental stimuli influence the expression of the tcpPH promoter; and 4) to determine the importance of AphB-mediated differential activation of tcpPH expression for pathogenesis in vivo. These studies will enhance our understanding of the complex mechanisms utilized by pathogenic bacteria to regulate virulence gene expression in response to environmental stimuli so that better strategies can be developed to control and prevent bacterial infections.

**Grant:** 2R01AI041699-07  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** ROY, CRAIG R PHD MICROBIOLOGY &  
IMMUNOLOGY  
**Title:** Genetic Analysis of Legionella Phagosome Trafficking  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 1997/07/01-2007/07/31

DESCRIPTION (provided by applicant): The respiratory pathogens *Mycobacterium tuberculosis*, *Chlamydia pneumoniae*, and *Legionella pneumophila* all have an obligate intracellular lifestyle that requires establishment of a replicative vacuole that does not fuse with lysosomes. Despite the fact that creation of a non-degradative vacuole is an essential virulence trait, very little is known about the molecular mechanisms that govern biogenesis of the replicative organelles in which these bacteria reside. *L. pneumophila* has become a valuable model organism for identifying bacterial determinants that govern biogenesis of these specialized replicative organelles. The Dot/Icm (defective organelle trafficking, and intracellular multiplication) transporter is a protein secretion apparatus that is essential for *L. pneumophila* pathogenesis. This transporter has the ability to inject bacterial proteins into eukaryotic host cells. These proteins are predicted to regulate the formation of a vacuole that supports intracellular growth of *L. pneumophila*. The goal of this grant is to understand how this transporter functions. Towards this end we will identify bacterial and host factors that play important roles in biogenesis of a replicative vacuole. Specifically, we will: 1) identify and investigate functional domains in cytoplasmic Icm proteins that regulate distinct activities necessary for biogenesis of a replicative organelle, 2) investigate the role of DotA protein secreted by the Dot/Icm transporter, 3) characterize RalF protein, which is an ARF-specific guanine nucleotide exchange factor injected into host cells by the Dot/Icm transporter, 4) identify other substrates of the Dot/Icm transporter that are injected into host cells, 5) define cellular processes that are required for the establishment of a replicative organelle by *L. pneumophila*. Determining the molecular function of *L. pneumophila* gene products required for phagosome trafficking will provide us with a foundation for how bacterial pathogens are able to alter their intracellular fate and profoundly affect the development of new strategies for fighting diseases caused by bacteria that modify phagosome maturation.

**Grant:** 2R01AI042143-05  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** LUKEHART, SHEILA A  
**Title:** Treponema pallidum:Pathogenesis-associated molecules  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1997/12/01-2006/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): The pathogenic treponemes are closely related genetically but they cause clinically distinct diseases: *T. pallidum* subsp. *pallidum* causes venereal syphilis, with frequent invasion of the central nervous system and infection of the fetus; *T. pallidum* subsp. *pertenue* and *T. pallidum* subsp. *endemicum* cause the nonvenereal yaws and bejel, respectively, which do not invade the CNS or infect the fetus in utero. *T. paraluiscuniculi* causes venereal disease in rabbits but is not infectious for humans. The old syphilis literature tells us that infection-derived immunity is complete for homologous strains but is partial or nonexistent between subspecies or species of pathogenic treponemes. Therefore, any antigenic epitopes shared with other subspecies or species of *Treponema* are unlikely to be protective. We propose to exploit those genetic and antigenic differences as a tool to identify subspecies- and species-unique genes and molecules that are most likely to play a role in immunity and pathogenesis. New molecules will be identified by immunological selection of antigens from *T.p. pertenue* and *T. paraluiscuniculi* expression libraries using specific antisera (Aim 1). Genetic and antigenic differences have already been identified within the *tpr* gene family, and these genes/antigens will be further explored in the non-pallidum treponemes (Aim 2). For selected genes, expression will be examined by quantitative RT-PCR (Aim 3). Purified recombinant molecules will be used for immunological studies defining species- or subspecies-specificity and development of immunity during infection (Aim 4); ability to induce protective immunity against homologous and heterologous challenge will be examined (Aim 5). This approach will lead to the identification of molecules that are critical to the pathogenesis of, and protective immunity to, treponemal infections.

**Grant:** 2R01AI043060-05  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** CAMPBELL, LEE A PHD MICROBIOLOGY, OTB  
**Title:** Chlamydia pneumoniae Antigens of Biological Significance  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1998/04/01-2007/03/31

DESCRIPTION (provided by the applicant): Chlamydia pneumoniae is a human respiratory pathogen that causes 5 percent to 10 percent of pneumonia, bronchitis, and sinusitis. Virtually everyone is infected in his or her lifetime and reinfection is common. Infection is difficult to treat even with sensitive antibiotics. Chronic infection is common and has been associated with asthma, reactive airway disease, Reiter's syndrome, erythema nodosum, and sarcoidosis. The potential public health impact of infection with this pathogen is underscored by the association of C. pneumoniae with atherosclerosis and related clinical manifestations such as coronary heart disease, carotid artery stenosis, aortic aneurysm, claudication, and stroke. If C. pneumoniae infection plays a role in atherogenesis, there will be an urgent need to facilitate diagnosis and develop strategies for intervention and prevention. The overall goal of this proposal is two fold. First, C. pneumoniae specific antigens that are recognized during human infection will be exploited to facilitate serodiagnosis and identify putative vaccine candidates. The second goal is to define chlamydial/host cell interactions that lead to entry and survival of C. pneumoniae in host cells relevant to atherosclerosis. The specific focus will be on the interaction of the chlamydial glycan moiety with carbohydrate binding receptors on the host cell. Importantly, infection of epithelial cells can be inhibited with N-linked high mannose type oligosaccharide, the major component of the glycan. The novel hypothesis to be tested is that C. pneumoniae enters through the mannose-6 phosphate receptor by binding to the site involved in transport of phosphomannosylated residues to the lysosome and this differs from C. trachomatis, which utilizes the mannose receptor. The ultimate goals of these studies are to identify C. pneumoniae specific antigens to facilitate laboratory diagnosis and virulence factors playing a role in pathogenesis to guide vaccine development or develop anti-adhesive strategies for prevention of infection.

**Grant:** 2R01AI043363-05  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** MOBLEY, HARRY L PHD MICROBIOLOGY, OTTAWA  
**Title:** Molecular Pathogenesis of E. Coli UTI  
**Institution:** UNIVERSITY OF MARYLAND BALTIMORE PROF SCHOOL BALTIMORE, MD  
**Project Period:** 1998/09/01-2007/08/31

DESCRIPTION (provided by applicant): Urinary tract infection (UTI) is the most frequently diagnosed kidney and urological disease and E. coli is by far the most common etiologic agent. E. coli strains associated with cystitis and acute pyelonephritis often express secreted proteins and multiple fimbrial types which are thought to contribute to their ability to colonize the urinary tract, persist at this site, and elicit overt and recurrent disease. E. coli CFT073, a representative and highly virulent pyelonephritis strain, isolated by our group, will be used to study a secreted autotransported toxin, Sat, along with six other apparent autotransporter homologs; and the role in virulence of the invertible element-mediated phase variation of type 1 fimbrial gene expression. The specific aims of the study are: 1) to determine the mechanism of action and role in virulence of the Sat cytotoxin secreted by uropathogenic E. coli; and 2) to determine the contribution to virulence in the urinary tract of invertible element-mediated phase variation of type 1 fimbriae. We propose to characterize the Sat protein with respect to secretion, protease activity, internalization into the host cell, and toxin activity in vivo in the CBA mouse model of UTI. Six additional autotransporter genes have also been identified in the genomic sequence of strain CFT073 and will be characterized with respect to cytopathic or cytotoxic activities. With respect to type 1 fimbriae phase variation, we will construct locked-ON and locked-OFF mutants and assess the contribution to virulence of this critical adhesin both in the bladder and in the kidney. The long-term goal of this proposal is to understand the role of cytotoxins and adhesins in the virulence of uropathogenic E. coli in the urinary tract and to develop preventative strategies based on our understanding of the mechanism of this organism's pathogenesis.



**Grant:** 2R01AI044193-04  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** COMSTOCK, LAURIE E  
**Title:** Regulation of Abscess Formation by *Bacteroides fragilis*  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 1998/12/01-2007/01/31

*Bacteroides fragilis* is the leading cause of anaerobic bacteremia and intraabdominal abscesses. Studies have shown that the interaction of the *B. fragilis* capsular polysaccharide complex (CPC) with the host immune system leads to the formation of intraabdominal abscesses. The *B. fragilis* CPC is composed of at least five distinct capsular polysaccharides, polysaccharide A, B, C, D, and E (PS A-E). Each of these capsular polysaccharides is able to undergo a reversible ON/OFF phenotype known as phase variation. The biosynthesis loci containing the genes for the synthesis of PS A, PS B and PS C have been completely sequenced as well the region upstream of the PS D locus. These sequences have allowed for the analysis of the mechanism phase variation of these capsular polysaccharides at the genetic level. Preliminary data suggest that variation of the *B. fragilis* capsular polysaccharides occurs due to the DNA inversion of a small segment of DNA upstream of the respective polysaccharide biosynthesis locus. The invertible regions upstream of the PS A and PS D loci have been demonstrated to contain functional promoters. This application is divided into aims that will analyze phase variation of these capsular polysaccharides at the DNA, RNA and polysaccharide levels. Studies will be conducted to characterize the cis-acting elements necessary for DNA inversion of promoter regions and the trans-acting factors that mediate inversion. Additionally, the characteristics of phase variation of each of these capsular polysaccharides will be analyzed from organisms grown in vitro, and isolated from in vivo sites associated with health (colon) and disease (peritoneal cavity and abscesses). Due to the novelty of the phase variation of five distinct capsular polysaccharide by a single organism, we have the unique opportunity to determine if there is coordinate regulation between the expression of distinct capsular polysaccharides, and the role phase variation of the *B. fragilis* capsular polysaccharides may play in health and disease.

<b>Grant:</b>	2R01AI044854-04	
<b>Program Director:</b>	MILLER, MARISSA A.	
<b>Principal Investigator:</b>	WALKER, SUZANNE L	PHD
<b>Title:</b>	Structural and Mechanistic Studies on MurG	
<b>Institution:</b>	PRINCETON UNIVERSITY	PRINCETON, NJ
<b>Project Period:</b>	1999/03/01-2007/02/28	

DESCRIPTION (provided by applicant): The specific aims in this grant are directed towards understanding the structure and mechanism of an essential bacterial glycosyltransferase that is involved in the biosynthesis of peptidoglycan. This enzyme, MurG, is found in all organisms that synthesize peptidoglycan and is a target for the design of new antibiotics. We developed the first direct assay to study MurG and recently solved the crystal structure of E. coli MurG. This first MurG structure, combined with sequence data on other glycosyltransferases, has revealed that MurG is a paradigm for a large family of metal ion-independent glycosyltransferases found in both eukaryotes and prokaryotes. Therefore, the studies proposed here are not only relevant to understanding an important antibacterial target, but will shed light on the structure and mechanism of an entire class of glycosyltransferases. Our long term goals are to learn how to design inhibitors for this family of glycosyltransferases and to learn how to manipulate substrate specificity. The former goal could lead to the development of new drugs; the latter should facilitate efforts to understand the cellular roles of glycosyltransferases and their products.

**Grant:** 2R01AI045842-04  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** PALZKILL, TIMOTHY G PHD  
**Title:** FUNCTIONAL GENOMICS STUDY OF TREPONEMA PALLIDUM  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 1999/07/15-2007/02/28

DESCRIPTION (provided by applicant): The genome sequences of many microorganisms have now been determined. Several of these organisms are the agents of infectious disease. The sequences reveal many genes of unknown function. The genome sequence information should enable new approaches to be developed to determine the function of genes and their possible role in pathogenesis. A functional genomics approach will be used to identify proteins important for the *Treponema pallidum* host-pathogen interaction. *T. pallidum* is the causative agent of syphilis. The complete genome sequence of this organism has been completed. Several features of *T. pallidum* make it an excellent system on which to develop and test functional genomics technologies. First, with a size of 1 million base pairs, the genome is one of the smallest known. Second, there are a total of 1031 open reading frames, which makes it feasible to systematically construct libraries containing each open reading frame in a relatively short period of time. Finally, little is known of the biology or pathogenesis of this organism because a continuous culture system is not available. This severely limits the experimental options for study of the organism. Therefore, new approaches are needed to understand gene function in *T. pallidum*. During the previous funding period, we have used a topoisomerase-based method to clone PCR products encoding 1008 of the 1031 open reading frames identified in the genome sequence of *T. pallidum*. In addition, the plasmid vector system used for cloning the open reading frames, the univector system, permits the rapid conversion of the original plasmid clone set to other functional vectors containing various promoters or tag sequences. The conversion to functional vectors is based on a single step Cre-loxP site-specific recombination reaction. Using Cre-loxP recombination, the *T. pallidum* clone set has been converted to specialized vectors for large scale protein expression, phage display and two-hybrid analysis. These plasmid collections will be used in a functional genomics approach to i) identify proteins involved in adhesion to host cells, ii) systematically identify *T. pallidum* antigenic proteins, and iii) establish a large-scale protein-protein interaction network among periplasmic and surface localized proteins.

**Grant:** 1R01AI046430-01A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** SANDOR, MATYAS PHD  
**Title:** Mycobacterial antigen compartmentalization & immunity  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2002/04/01-2006/03/31

Tuberculosis remains one of the most significant public health challenges the modern world faces. Improved vaccines are needed for prevention of infection and improved immunotherapies are needed to combat existing or recurring disease. A better vaccine would induce a concerted protective response by CD4+ and CD8+ T cells together. Our hypothesis is that CD4+ and CD8+ T cells sample different bacterial compartments differently. Information about which compartment is optimal for effective presentation of Class II and Class I epitopes and generation of protective responses will help create better vaccines. To do this we will use a novel approach while building on the strengths of previous experimental systems. TCR transgenic mice will be infected with recombinant Mycobacterium bovis strain bacille Calmette Guérin (BCG), the current vaccine strain. These rBCG will express T cell epitopes in the context of the same fusion proteins located in different subcellular compartments of the bacteria. A parallel series of rBCG strains will be constructed for both class I or class II presentation using either Lymphochoriomeningitis Virus (LCMV) gp33 peptide or pigeon cytochrome C peptide (PCC) respectively. We will study how access of each epitope to its respective presentation pathway is influenced by its location in different bacterial compartments. Subsequently, we will study the activation and recruitment of antigen specific cells both systemically and in the BCG induced liver granulomas in response to various rBCG using adoptively transferred antigen specific T cells. Our final analysis will be to study how bacteremia is effected when antigen is presented in different bacterial compartments with or without prior peptide specific immunization. In this manner we hope to define how the different epitopes in different bacterial compartments effect T cell responses and protection. We chose PCC (CD4+ specific) and gp33 (CD8+ specific) for this work because they are both widely studied model antigens and a multitude of reagents are available, including T cell clones, hybridomas, TCR transgenic mice, and MHC tetramer reagents. The mouse model of BCG infection was chosen because we wish to improve the vaccine capacity of this attenuated strain and also because infection of mice with BCG has been widely employed and many of the characteristics of this model are well understood. The experimental results from this proposal should have direct relevance to improving vaccine design for protection against tuberculosis, and will also provide knowledge about how bacterial antigen access different antigen presenting pathways.

**Grant:** 1R01AI046512-01A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** SMITH, ARNOLD L MD PEDIATRICS:INFECTIO  
DISEASES  
**Title:** Virulence genes Haemophilus influenzae  
**Institution:** UNIVERSITY OF MISSOURI COLUMBIA COLUMBIA, MO  
**Project Period:** 2002/09/15-2002/09/16

DESCRIPTION (provided by applicant): Haemophilus influenzae is a human-restricted bacterium which can exist as an upper respiratory commensal, but also cause focal and/or systemic disease. More than 95 percent of the nasopharyngeal isolates lack capsules and are not serotypeable, and cause focal infections, while encapsulated (and rare nontypeable) strains cause sepsis and meningitis. The genome of both commensal and pathogenic isolates is 250 kb (on average) larger than the avirulent laboratory strain (Rd KW2O) whose genome sequence was published in 1995. We have found that the genome of strain Rd KW2O is a "scaffold" on which genes, gene clusters and operons encoding virulence factors are inserted, usually at the Haemophilus-specific DNA uptake sites. Using differential hybridization we will identify those DNA loci that permit nasopharyngeal colonization by commensals, and the additional putative virulence loci in pathogenic isolates. Each of these DNA fragments and the flanking DNA, will be sequenced until the Rd KW2O scaffold is identified. All sequence information will be posted on the UW Genome Center web site. Using strict criteria for homology (not conventional criteria) putative virulence genes will either be deleted or mutated and the virulence of the mutant compared to the parent in a relevant in vitro and/or in vivo infection model. For example a putative adhesin present in a lower respiratory tract isolate could be tested with human respiratory epithelial cell lines, and with primary human respiratory epithelium growing at an air-liquid interface in tissue culture. One unique isolate, an untypeable strain causing meningitis in an immunocompetent child immunized with a Hib-conjugate vaccine appears to be a member of a clade; we wish to confirm the preliminary finding and initiate a global database (in collaboration with the MLST Haemophilus Center) for these vaccine-failure isolates. With an understanding of the molecular mechanisms of colonization versus infection, strategies for prevention and intervention can be devised.

**Grant:** 1R01AI047142-01A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** OWEN, WHYTE G PHD BIOCHEMISTRY  
**Title:** KatG Peroxidase in Isoniazid Activation and Resistance  
**Institution:** MAYO CLINIC COLL OF MEDICINE, ROCHESTER, MN  
ROCHESTER  
**Project Period:** 2002/05/01-2006/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): Tuberculosis is one of the leading causes of death worldwide from an infectious disease. Isoniazid is a front line antibiotic used in the treatment of tuberculosis. The Mycobacterium tuberculosis heme catalase/peroxidase KatG is responsible for activating isoniazid to a reactive biocidal species. Antibiotic resistance to isoniazid is a growing concern and can occur by deletions or point mutations in the katG gene. One of these, a serine-to-threonine substitution at position 315, KatG(S315T) is found in about 50 percent of clinical isolates resistant to isoniazid. Our research seeks to understand the mechanism of isoniazid activation and molecular basis for drug resistance caused by the S315T and other point mutations in KatG using biochemical, genetic, and spectroscopic techniques. KatG will be purified from recombinant and native sources and the activities and spectroscopic properties compared. Besides a catalase/peroxidase activity, KatG can also catalyze several other reactions including Mn<sup>2+</sup> peroxidase, P450-like oxygenase, and peroxynitritase activities. Which of these or possibly other redox reactions are responsible for isoniazid oxidation and activation will be tested using wild-type and mutant KatG proteins to investigate the mechanism of activation. Genetic methods investigating the role of superoxide in isoniazid activation are proposed. Optical, EPR, NMR, and resonance Raman spectroscopies are revealing subtle differences in the heme active site of wild-type KatG and KatG(S315T). These spectroscopic techniques will be applied to various forms of wild-type and mutant enzymes in order to elucidate the molecular basis for reactivity toward isoniazid and the reduced rate of isoniazid oxidation by KatG(S315T). NMR relaxation measurements and x-ray crystallography will be used to map the isoniazid binding site on both enzymes to determine whether subtle differences in distance and/or orientation are responsible for the reduced turnover of drug by the mutant enzyme. Steady-state and rapid kinetic techniques will follow ligand-binding rates to the heme iron and the formation and decay of reactive intermediates in the catalytic cycle for both wild-type KatG and KatG(S315T) to determine whether the S315T mutation affects one of the steps in the catalytic cycle. EPR spectroscopy and spin-traps are being used to trap reactive intermediates. The stable products formed in this reaction are being characterized by mass spectrometry to reveal chemical information about the nature of intermediates in the reaction of isoniazid oxidation by KatG. Site directed mutagenesis of residues also implicated in isoniazid resistance other than S315 will be generated and the effects on enzyme activities and spectroscopic properties examined to determine structural information about the mutant enzymes. Analogs of isoniazid will be used as additional biochemical and spectroscopic probes of the reaction mechanism and molecular basis for drug resistance.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R01AI047744-01A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** SHERMAN, DAVID R PHD  
**Title:** Hypoxia, Latency and Reactivation in M.tuberculosis  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/09/15-2007/02/28

DESCRIPTION (Provided by the applicant): Central to the pathogenic success of Mycobacterium tuberculosis (MTB) is its ability to persist within humans for long periods in a latent state, without causing any overt disease symptoms. Roughly one-third of the world population harbors latent MTB, greatly complicating efforts at tuberculosis control. A person with latent tuberculosis has about a 10 percent lifetime chance of developing active disease, and when such a person contracts HIV, the risk of developing reactivation TB increases to 8 - 10 percent per year. Hypoxic conditions within the human host are widely regarded as crucial for development of latent tuberculosis, but the MTB adaptive response to hypoxia is at present very poorly understood. The goal of this proposal is to define the MTB hypoxic response as it relates to latency and reactivation. We will mechanistically dissect this response and analyze the role of hypoxia in latent tuberculosis and reactivation. This proposal will define the genes whose response to reduced oxygen tension comprises the MTB hypoxia regulon. We will also focus on MTB alpha-crystallin (Acr), a component of the hypoxic response that is powerfully induced by microaerophilic conditions. We will determine the specific conditions in which expression of alpha-crystallin and its regulators is necessary for achieving latency or reactivation. Finally, we will dissect the alpha-crystallin regulatory machinery to determine the precise mechanisms by which oxygen tension controls MTB gene expression. The result will be better tools to confront the threat to more than one billion persons with latent tuberculosis, millions of whom are now or will soon be co-infected the the human immunodeficiency virus, HIV.



**Grant:** 1R01AI047866-01A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** CIRILLO, JEFFREY D  
**Title:** Entry Mechanisms of Mycobacterium marinum  
**Institution:** UNIVERSITY OF NEBRASKA LINCOLN LINCOLN, NE  
**Project Period:** 2002/09/15-2007/02/28

DESCRIPTION (provided by the applicant): Mycobacterium marinum is the causative agent of localized lesions in the extremities, commonly called swimming pool or aquarium granuloma. Swimming pool granuloma is relatively common in professions involving almost any water source, including aquarium maintenance, fishing and diving. Currently, infections occur at a frequency of nearly 200 per year in the U.S. alone and are thought to be on the rise. Improved methods for disease prevention are a high priority, since antibiotic therapy usually requires multiple drugs and from four to fourteen months before full recovery. M. marinum replicates primarily intracellularly in macrophages during disease in humans. Thus, M. marinum must enter a eukaryotic cell in order to replicate and the genes involved in entry should be critical for production of disease. Our previous studies have resulted in the discovery of two key aspects of the ability of M. marinum to invade host cells. First, we have found that M. marinum enters host cells at up to 100 fold higher levels than the non-pathogenic mycobacterial strain M. smegmatis. This entry mechanism appears to enhance the ability of M. marinum to survive intracellularly. Second, the ability of M. marinum to enter host cells is regulated by growth conditions. We have utilized these data to develop novel strategies for isolation of the genes required for entry of M. marinum into host cells. The close genetic relationship of M. marinum to other mycobacterial species such as M. avium and particularly M. tuberculosis, suggests that the genes identified may play a similar role in other pathogenic mycobacteria. Our hypothesis is that the ability to enter host cells is important for virulence of mycobacteria. The specific aims of the current proposal are: 1) isolate and characterize mycobacterial genes involved in entry and 2) determine the involvement of these genes in virulence in mice. Through an examination of the mechanisms of entry and the factors that regulate it, we hope to further our understanding of how mycobacteria cause disease as well as provide insight into novel methods for their prevention.

**Grant:** 1R01AI047880-01A2  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** TOTTEN, PATRICIA A  
**Title:** Hemolysin and Immunobiology of Chancroid  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/09/30-2005/03/31

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

**Grant:** 1R01AI047900-01A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** HASLAM, DAVID B MD  
**Title:** ER-Localized Chaperones in Toxin Pathogenesis  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2002/08/15-2007/01/31

DESCRIPTION (provided by applicant): Shiga toxin follows a complex intracellular pathway in order to kill susceptible cells. After binding to cell surface glycolipids, the toxin is internalized and trafficked in retrograde fashion to the Golgi and endoplasmic reticulum (ER). From the ER lumen, the toxin must gain access to the cytoplasm, where it enzymatically inactivates the 28S ribosomal RNA, inhibiting protein synthesis. This recently discovered pathway from the endoplasmic reticulum lumen to the cytoplasm represents a cellular quality control mechanism responsible for degrading host proteins that misfold in the ER lumen (termed ER-associated degradation, or ERAD). Shiga toxin apparently "pretends" to be a misfolded host protein, and thereby utilizes the quality control pathway to gain access to ribosomes in the cytoplasm. In yeast, ERAD is known to require a pore called Sec61, as well as chaperones within the ER lumen. The host molecules involved in this pathway in higher eucaryotes are largely unknown. Recently, however, cholera toxin was found to be transported from the ER through Sec61. We devised a genetic screen for molecules involved in shiga toxin trafficking and isolated a cDNA encoding a novel chaperone. This chaperone, which we named HEDJ, was found to be localized to the ER lumen and to interact with Bip, a molecule known to be involved in protein translocation out of the ER. We demonstrate here that HEDJ interacts with shiga toxin in the ER lumen prior to toxin transport. We propose that STx, cholera and other toxins co-opt HEDJ and other chaperones for transport across the ER membrane. However, unlike host molecules transported in this manner, we propose that STx bypasses proteosomal degradation within the cytosol. Using in vitro assays developed in our laboratory, the experiments proposed here will address the role of HEDJ and other chaperones in toxin translocation to the cytoplasm. Sequential co-immunoprecipitation experiments will allow a detailed dissection of the ER-localized chaperones involved in toxin transport. Turning our attention to the toxin, structure-function analyses will determine which portion(s) of STx enables the molecule to interact with chaperones and exit the ER. Additionally, we will determine whether STx avoids proteosomal degradation and will begin to examine the mechanism of proteosomal avoidance.

**Grant:** 1R01AI047932-01A2  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** STICH, ROGER W PHD  
**Title:** Transmission of Ehrlichia chaffeensis by Adult Ticks  
**Institution:** OHIO STATE UNIVERSITY COLUMBUS, OH  
**Project Period:** 2002/08/01-2007/05/31

DESCRIPTION (provided by the applicant): Approaches to the control of human monocytic ehrlichiosis (HME), the most prevalent zoonotic ehrlichial disease reported in the USA to date, rely on better understanding of interactions between Ehrlichia chaffeensis, the etiologic agent, and its tick host. It has been demonstrated that several ixodid genera, including Amblyomma, Dermacentor and Ixodes, can be naturally infected with E. chaffeensis. Although detection of E. chaffeensis in naturally exposed ticks indicates susceptibility of these ticks to infection, it does not demonstrate their ability to transmit this pathogen to a susceptible vertebrate host. Repeated experimental transmission of an arthropod-borne pathogen by the suspected vector(s) is chief among the criteria that must be fulfilled before the competent vector(s) is (are) incriminated. The objective of this particular application, which is an essential step in determining the mechanisms behind tick transmission of E. chaffeensis, is to identify tick species and pathogen-acquisition scenarios that lead to effective experimental transmission of this pathogen. The central hypothesis of this project is that intrastadially infected adult male ixodid ticks are more effective experimental vectors of E. chaffeensis than adult female or trans-stadially infected adult male ticks. This central hypothesis will be tested through fulfillment of specific aims focused on (1) development of a PCR assay for E. chaffeensis that is sensitive enough to detect the pathogen within individual experimentally infected ticks, (2) intrastadial and transstadial tick-transmission of E. chaffeensis and (3) transmission of E. chaffeensis to multiple hosts by adult male ticks. The rationale for the proposed research is that it will lead to better understanding of the potential importance of different tick species and acquisition scenarios in the transmission of E. chaffeensis under natural conditions. Furthermore, once effective transmission cycles are identified, an experimental transmission model will be available to enable us to investigate interactions between E. chaffeensis and its tick host(s). This work is innovative because it questions the dogma that this tick borne pathogen is only transmitted transstadially, but this paradigm will be tested objectively with both intrastadial and transstadial transmission scenarios. In addition to development of a transmission model, the competence of several potential vectors will be determined, which is important for determining whether dogs could be a source of human infection with E. chaffeensis and for accurate diagnosis and prevention of HME. Collectively, these outcomes are expected to have significant positive effects on human (and animal) health, because they will lead to understanding the mechanisms responsible for transmission of E. chaffeensis and perhaps other closely related pathogens among human and domestic hosts.

**Grant:** 1R01AI047958-01A2  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** DILLARD, JOSEPH P  
**Title:** Type IV secretion by *Neisseria gonorrhoeae*  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2002/07/01-2006/06/30

DESCRIPTION (provided by applicant): *Neisseria gonorrhoeae* causes the sexually transmitted disease gonorrhea, the more serious infections pelvic inflammatory disease (PID) and disseminated gonococcal infection (DGI). We identified a 57 kb genetic island present in *N. gonorrhoeae* that shows the characteristics of a pathogenicity island. Several forms of the gonococcal genetic island (GGI) are present among disease isolates, with approximately 80 percent of gonococcal strains carrying some form of the GGI. We identified a peptidoglycan hydrolase (*atlA*) encoded in the GGI and found that it is involved in the production of an unusual cytotoxin derived from the bacterial cell wall. This peptidoglycan-derived cytotoxin (PG-cytotoxin) is identical to the tracheal cytotoxin of *Bordetella pertussis* and is an important virulence factor in gonococcal infections. PG-cytotoxin causes the death of ciliated fallopian tube cells in organ culture, induces arthritis in rats, and induces IL-1 and IL-6 in cultured cells. The presence of *atlA* in the genetic island is significantly correlated with strains that cause DOT. DNA sequencing revealed that the GGI encodes a putative type IV secretion system. Type IV secretion systems include plasmid conjugation systems and virulence factor export systems and some type IV secretion systems carry out both processes. Mutations in the putative type IV secretion genes in the GGI result in loss of DNA secretion. These mutants also show delayed adherence to primary cervical cells and exhibit an unusual microcolony phenotype upon binding to cells. Mutations in *atlA* result in the same phenotypes as the other type IV secretion mutations as well as showing decreased PG-cytotoxin production. Thus *AtlA* is necessary for type IV secretion and is either directly or indirectly involved in PG-cytotoxin production. These results suggest that the GGI encodes an active type IV secretion system important in interaction of *N. gonorrhoeae* with the host. The goals of this proposal are contained in two specific aims. 1) We will make mutations designed to affect type IV secretion and test these mutants for secretion of proteins, DNA, and PG-cytotoxin. 2) We will test mutants with defects in secretion for phenotypes relevant to virulence, i.e., epithelial cell adherence and invasion, intracellular survival, and microcolony formation. Overall, these studies are designed to reveal the molecular mechanisms of this novel secretion system and identify previously unrecognized virulence factors important in gonococcal infection which may serve as new targets for chemotherapy or immunoprotection.

**Grant:** 1R01AI048616-01A2  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** LAZAZZERA, BETH A BS  
**Title:** Mechanism of Peptide Signaling in Bacillus Subtilis  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2002/03/01-2007/02/28

DESCRIPTION (provided by applicant): The long-term goal of this proposal is to understand a novel mechanism of peptide signaling in Gram-positive bacteria using Competence and Sporulation Factor (CSF) of *Bacillus subtilis* as a model system. CSF is a member of an emerging class of signaling peptides that function intracellularly. Previously, signaling peptides had been thought to function only through membrane receptors, due to the presence of peptidases inside the cell. However, our studies have shown that CSF, an unmodified five amino acid peptide, is transported into the cell where it functions intracellularly to regulate gene expression. Peptide signals, like CSF, are used by Gram-positive bacteria to monitor their population density (i.e. quorum sensing). Quorum sensing in bacteria regulates such medically important processes as virulence and biofilm development. Therefore, functions regulated by peptide signaling pathways in the medically important Gram-positive bacteria are potential drug targets. We hope that, by understanding the mechanism of signaling by CSF in *B. subtilis*, we will be able to identify additional intracellular functioning signaling peptides in other bacteria. We have proposed experiments to understand the mechanism of production and response to CSF. The precursor protein for CSF is secreted through the general Sec-dependent export pathway and then processed extracellularly. We have isolated a mutant that appears to be defective in this extracellular processing activity. We have proposed genetic and biochemical experiments to characterize the role of protein defective in this mutant in CSF production. Mature CSF is imported by a non-specific oligopeptide permease (Opp), homologues of which exist in many bacteria. Experiments are proposed to determine the binding affinity of CSF for Opp and whether other peptides compete with CSF for binding to Opp. Once inside the cell, CSF appears to have at least three intracellular receptors. We have proposed experiments to identify the intracellular receptors of CSF and to understand how CSF interacts with these receptors. CSF regulates the activity of two phosphatases that dephosphorylate response regulators. These phosphatases are intriguing as they show similarity to the widespread tetratricopeptide repeat (TPR) protein domain family. The proposed experiments should elucidate the mechanism of signaling by this novel class of intracellular function peptides.

**Grant:** 1R01AI048634-01A2  
**Program Director:** SAVARESE, BARBARA M.  
**Principal Investigator:** TOTTEN, PATRICIA A  
**Title:** Clinical Epidemiology of Mycoplasma genitalium  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/03/01-2007/02/28

Large proportions of the major reproductive tract inflammatory syndromes remain idiopathic, not attributable to the major sexually transmitted pathogens such as *Chlamydia trachomatis* or *Neisseria gonorrhoeae*. Where effective STD control programs exist, most urethritis in men and endocervicitis or mucopurulent cervicitis (MPC) in women is no longer attributable to gonococcal or chlamydial infection. This is equally true for most upper genital tract complications of urethritis (epididymitis) or endocervicitis (endometritis, salpingitis and perinatal and puerperal morbidity). *Mycoplasma genitalium*, a fastidious bacterium discovered in 1981, now detectable by PCR, has been significantly associated with nongonococcal urethritis (NGU) in men in 11 of 11 studies over the past decade using PCR, including our own recent study which demonstrated *M. genitalium* in 27 (22%) of 211 men with and 5 (4%) of 117 without NGU (OR 6.5; 95% CI 2.1- 19.9). Recognition of *M. genitalium* as a pathogen in the male raises the important question of its role as a pathogen in the female, both in nonpregnant and in pregnant women. Since initial submission of this proposal in February 2000, we have completed two retrospective cross-sectional studies involving women. In a random sample of female STD clinic patients, we demonstrated endocervical *M. genitalium* infection in 24 (13%) of 191 with MPC vs. 27 (6%) of 453 without MPC (OR adjusted for cervical pathogens 3.0; 95% CI 1.6-5.8). This study also detected *M. genitalium* in 10 (14.3%) of 70 women with history of spontaneous miscarriage at < 20 weeks gestation vs. 41 (7.2%) of 570 without this history (adj OR=2.5; 95% CI 1.1-5.6). A cross-sectional study of 115 Kenyan women with suspected PID demonstrated *M. genitalium* in endometrial biopsies from 7 (12%) of 58 women with endometritis vs. 0 of 57 without endometritis (p=0.01). In our studies of male urethritis, MPC, and endometritis, associations of *M. genitalium* with disease were similar to, or stronger than, the associations with chlamydial infection. These data support our proposed studies as the next logical step in clinical epidemiologic studies of this pathogen. Our three specific aims are to (1) define the role of *M. genitalium* in acute salpingitis in women undergoing laparoscopy in Nairobi Kenya; (2) define the association of *M. genitalium* with abnormal pregnancy outcomes including preterm delivery of a low birthweight infant, using data and clinical specimens already available from 2500 women prospectively followed to term at University of Washington hospitals (including 625 with gestation <37 weeks); and (3) determine (a) risk factors for *M. genitalium* infection in a population-based sample of young women participating in Wave 3 of the National Longitudinal Study of Adolescent Health, and in a sample of higher risk women attending the Seattle STD clinic, and (b) concordance of *M. genitalium* infection in these women and their sex partners. *M. genitalium* may represent an important new pathogen in the female reproductive tract. Studies of its association with salpingitis and pregnancy morbidity are essential. Future studies should also address whether, similar to gonorrhea and chlamydial infection, it facilitates transmission of HIV infection.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials



**Grant:** 1R01AI048694-01A2  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** NIZET, VICTOR F MD MEDICINE  
**Title:** SLS: Molecular Basis and Role in Invasive GAS Disease  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2002/04/01-2007/03/31

DESCRIPTION (provided by applicant): Group A Streptococcus (GAS) is a major human pathogen producing invasive infections including necrotizing fasciitis (NF). The virulence factors responsible for bacterial spread and tissue injury in GAS NF are poorly understood. GAS are recognized phenotypically by a zone of beta-hemolysis produced largely by the cytolytic toxin streptolysin S (SLS). Our laboratory has led a collaboration that elucidated the genetic basis for SLS production. The 9-gene sag operon is both necessary for GAS SLS production and sufficient to confer SLS activity to the nonpathogenic heterologous species *Lactococcus lactis*. Sequence features and homologies strongly suggest SLS belongs to the bacteriocin class of toxins, with sagA encoding the toxin precursor (pre-SLS) and downstream genes (sagB-I) encoding chemical modification, processing and export functions. Target mutagenesis of each gene in the sag operon results in an SLS-negative phenotype. In vivo testing of SLS-negative sag knockout mutants in a mouse model of GAS NF showed that SLS is required for virulence. SLS-negative mutants failed to produce the necrotic ulcer, diffuse neutrophilic infiltrate, and widespread dermal and fascial tissue injury observed with the parent GAS strains. Our discovery and genetic analysis of the sag locus for SLS production has generated powerful information and reagents to study the molecular basis, biologic activities, and virulence properties of this GAS exotoxin. We hypothesize that each gene in the sag operon is required for proper expression of SLS, and that the SagA precursor is chemically altered, exported and processed to yield a mature protein with modified amino acids and structural features of a bacteriocin. We further hypothesize that GAS is a multifunctional toxin with cytotoxic and proinflammatory activities on host cells. Finally, we hypothesize that SLS plays an important role in the pathogenesis of GAS NF, through direct cytotoxicity, stimulation of neutrophil inflammation and interference with phagocytosis, perhaps acting synergistically with other GAS factors such as M-protein and SPE-B. These hypotheses will be tested by molecular genetic studies, attempts protein purification and antibody development, and the use of targeted SLS mutants in in vitro assays of phagocytic function and our in vivo mouse model of GAS NF.

**Grant:** 1R01AI048717-01A2  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** HODGES, ROBERT S PHD  
**Title:** Synthetic Peptide Consensus Sequence Vaccine Development  
**Institution:** UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO  
AURORA  
**Project Period:** 2002/08/15-2006/01/31

DESCRIPTION (provided by applicant): Hospital acquired infections such as *Pseudomonas aeruginosa* (PA) affect up to 10% of patients admitted to acute care hospitals, representing upwards of 10 million hospital days annually in North America. PA infection results in significant patient morbidity and mortality (ca. 80,000 deaths in North America per year due to PA ventilator-associated pneumonia in intensive care unit (ICU) patients). Patients considered being at risk of PA infections are those whose immune systems have been weakened because of accident, disease or other causes. PA infections are commonly found in patients with cancer, cystic fibrosis, AIDS, burn wounds or who have a long history of hospitalization. Although antibiotic therapy is employed in treatment it is often incapable of resolving the infection. This lack of effectiveness is due in part to antibiotic resistance of the bacteria, difficulty in establishing an exact diagnosis, and, paradoxically, antibiotic use which selects resistant bacteria. New approaches are needed to control infection which are based on prevention. The research described here is such an approach. Our laboratory has investigated PA infection for the last 10 years and has demonstrated the feasibility of preventing PA infection by using novel approaches to both active vaccination or by passive use of antibody therapeutics. Both these methods attack the infection process at its initial stage: attachment of the bacterium to the host's cell surfaces. However, the problem exists that there are multiple strains of PA bacteria and an efficacious vaccine or antibody therapeutic must account for all existing strains of this pathogen. In this proposal we are developing 1) a novel consensus sequence vaccine approach for coverage against all strains of PA, 2) a constrained peptidomimetic of the immunogen to enhance immunogenicity when used as an active peptide vaccine to block bacteria attachment and 3) monoclonal antibodies (prepared to selected peptide immunogens) that are broadly cross-reactive with maximal affinity for use as an antibody therapeutic.

**Grant:** 1R01AI048815-01A2  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** CHERAYIL, BOBBY J MD  
**Title:** Induction of Macrophage iNOS by Salmonella  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2001/12/01-2006/11/30

DESCRIPTION (provided by applicant): Various serotypes of the enteric Gram-negative bacterial pathogen *Salmonella* are responsible for a number of diseases of public health significance, including acute gastroenteritis, as well as typhoid fever. During the course of infection, these organisms invade intestinal epithelial cells, dendritic cells and macrophages of the host. In doing so, the bacteria introduce specific effector proteins into the host cells through a specialized secretory apparatus. The cytoskeletal changes and activation of cellular signaling pathways induced by these proteins facilitate bacterial invasion and also elicit the production of host pro-inflammatory molecules. Elucidating exactly how the effector proteins carry out these functions would help to clarify the pathogenesis of, and might suggest new approaches to treating, *Salmonella* -associated disease. In preliminary experiments, I have found that the effector SopE2, a guanine nucleotide exchange factor for mammalian Rho GTPases, is necessary for the *Salmonella* -dependent upregulation of inducible nitric oxide synthase (iNOS), the enzyme that is responsible for controlling the production of nitric oxide (NO) in macrophages. The pro-inflammatory and immunomodulatory effects of NO contribute to anti-microbial defense, as well as to the tissue damage that is associated with infection. In further studies, I have found that SopE2 activates the transcription factor NF-kappa-B, both on its own, and in a synergistic interaction with TRAF6, an adaptor molecule involved in signaling via members of the Toll-like receptor, and TNF receptor families. The experiments proposed in this application will extend these preliminary observations to elucidate the function of SopE2, and its homolog SopE, by (a) examining the mechanism by which SopE2 and SopE initiate signals leading to iNOS induction, particularly the role of the Rho GTPases in this process (b) elucidating how SopE2 and SopE activate NF-kappa-B, and characterizing their influence on TRAF-dependent signals, (c) identifying cis-acting transcriptional regulatory elements in the iNOS promoter that respond to *Salmonella* infection, and to Sop-induced signals, and (d) examining the role of SopE2 and SopE in iNOS induction by *Salmonella* in primary macrophages and dendritic cells. The results of these studies will shed light on a novel function of SopE and SopE2 and will also improve understanding of the mechanisms that regulate iNOS expression.

**Grant:** 1R01AI049003-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** CASJENS, SHERWOOD R PHD  
**Title:** Comparative Genomics of Lyme Disease Spirochetes  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 2002/09/01-2005/06/30

DESCRIPTION (provided by the applicant): This project aims to obtain an understanding of the nature, variability and evolution of the unusual genome of the spirochete bacteria (genus *Borrelia*) that cause human Lyme disease. Many of the genes that are thought to encode host-interaction genes in *Borrelia* are encoded on the numerous extrachromosomal DNA elements (plasmids) that these bacteria carry. Most of these "plasmids" (21 different ones in the only isolate to be exhaustively studied) may be present in nearly all natural isolates, and so could be thought of as "mini-chromosomes." Current evidence indicates that at least ten of the linear plasmids of the North American Lyme agent bacteria, *Borrelia burgdorferi*, are in the midst of a "rapid evolutionary spurt," as is evidenced by the presence in the one individual studied in detail, of many recent duplicative rearrangements and mutationally decaying duplicates of genes that are thought to be important to the organism. This application proposes to compare the complete nucleotide sequences of the plasmids several *B. burgdorferi* isolates, a *B. garinii* and a *B. afzelii* isolate (causative agents of Lyme disease in Eurasia), and isolates of a very closely related species that does not cause Lyme disease (*B. bissettii*). In addition, the chromosome of *B. garinii* will be sequenced. This "comparative genomics" approach is a powerful way to begin to deduce which plasmid genes are important in causing Lyme disease as indicated by their conservation among Lyme causative isolates, to deduce other general similarities and differences among the species under study, as well as to understand the mechanisms involved in the "rapid evolutionary changes" mentioned above. A longer term objective is to use this information to study a larger panel of independent strain isolates to understand the nature of the plasmid gene pool in the Lyme disease *Borreliae*, as well as to understand the relative rates of the "rapid evolution" (above), plasmid spread, and bacterial clonal expansion in the *B. burgdorferi* population. These findings will impact the study of the molecular pathogenesis of Lyme disease in many ways.

**Grant:** 1R01AI049013-01A2  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** MORAN, CHARLES P PHD GENETICS:GENETICS  
OTHER  
**Title:** RNA Polymerase Sigma Factors in Streptococcus pyogenes  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 2002/03/15-2006/02/28

Streptococcus pyogenes (the group A streptococcus or GAS) is an important and common human pathogen. The diseases it causes range from self-limiting skin and throat infections, with, however, the potential for serious sequelae including rheumatic heart disease, acute glomerulonephritis, and possibly pediatric neuropsychological disorders, to severe invasive diseases like myositis and streptococcal toxic shock syndrome. Since single strains of the GAS seem to be able to cause most or all of these diseases, regulation of the expression of GAS genes in response to specific environmental differences within the host is probably key in determining the course of the infectious process. We propose here a new approach to learn more about control of gene expression in the GAS by the characterization of a new RNA polymerase (RNAP) secondary sigma factor needed to transcribe genes under different conditions and the characterization of the genes it regulates.

**Grant:** 1R01AI049193-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** PETERSON, KENNETH M PHD  
**Title:** Signal Trans. and Intestinal Colonization by V.Cholerae  
**Institution:** LOUISIANA STATE UNIV HSC SHREVEPORT SHREVEPORT, LA  
**Project Period:** 2002/03/01-2007/02/28

DESCRIPTION (provided by the applicant): Understanding the mechanisms by which mucosal pathogens such as *Vibrio cholerae* colonize the human intestinal mucosa is key to the rational development of live-attenuated vaccine derivatives capable of inducing protective immunity against cholera and other enteric diarrheal diseases. Current parenteral vaccination strategies for these infections are largely ineffective. Although many *V. cholerae* genes required for intestinal colonization have been identified, the molecular mechanisms by which the proteins they encode promote vibrio adherence to host tissue is poorly understood. The studies described in this research proposal represent an attempt to understand at the molecular level, the contribution of the *V. cholerae* accessory colonization factor AcfB and AcfC proteins to the intestinal colonization properties of this emerging human pathogen. AcfB is a 75 kDa inner membrane protein that belongs to a large family of signal transducing proteins involved in bacterial chemotaxis. *V. cholerae* acfB mutants display an altered motility phenotype using a swarm plate motility/chemotaxis assay and produce reduced levels of cholera toxin and toxin-coregulated pilus. AcfC is a 26 kDa periplasmic protein that closely resembles bacterial sulfate binding proteins involved in solute transport and bacterial chemotaxis. Mutations within acfC specifically interfere with the ability of *V. cholerae* to migrate toward a gradient of galactose-6-sulfate in a standard chemotaxis assay. This proposal outlines a series of experiments aimed at understanding in molecular detail the contributions of the AcfB and AcfC proteins to vibrio chemotaxis/intestinal colonization. The long-term goal of these studies is to understand the structure and function of AcfB and AcfC so that we can use the information regarding the properties of these two proteins in the development of improved methods for treating and preventing cholera/enteric infections. There are four specific aims in the present proposal: (1) chemotaxis/intestinal colonization/pilus production assays will be used to determine the features of AcfB that promote chemotaxis and/or pilus synthesis; (2) in vitro/in vivo model systems will define the features of AcfC required for binding galactose-6-sulfate and the contribution of this process to vibrio chemotaxis/intestinal colonization; (3) the infant mouse model of cholera infection and excised intestinal tissue will be used to determine the nature of the colonization defect in *V. cholerae* carrying mutations within acfBC genes; (4) recombinase-based in vitro expression technology (RIVET) will elucidate the role of AcfB in promoting maximal levels of pilus synthesis.

**Grant:** 1R01AI049194-01A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** KRAUSE, DUNCAN C  
**Title:** Mycoplasma pneumoniae Gliding Motility  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 2002/06/01-2006/05/31

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. More effective means of prevention and control of mycoplasma infections requires that the basic biological processes of these unique, cell wall-less prokaryotes be characterized in more detail. M. pneumoniae infections in humans are transmitted by aerosol, leading to colonization of host respiratory epithelium at the base of the cilia. M. pneumoniae cells move by gliding motility, which undoubtedly contributes to their ability to localize successfully to a nutritionally preferred site. Therefore, gliding motility probably constitutes a virulence factor, but the contribution of gliding to virulence has not been determined. Gliding motility is poorly understood in bacteria in general and in mycoplasmas in particular. Remarkably, no homologs to known motility genes, either gliding or otherwise, have been identified in the genome sequence of M. pneumoniae. Recent studies revealed that the loss of protein P30 in the M. pneumoniae cytoadherence mutant II-3 also results in an abnormal cell morphology and loss of gliding motility. However, the hemadsorbing revertant of this mutant, designated II-3R, remains non-motile, clearly distinguishing the multiple functions of P30 in adherence and motility. Loss of motility correlates with a difference in the primary sequence of the revertant P30 over a 16-amino acid region. This proposal focuses on structure-function analysis of P30 in the context of motility, assessment of the role of motility in virulence in hamster tracheal rings in organ culture, and identification and analysis of other M. pneumoniae genes associated with gliding motility. Derivatives of recombinant P30 will be constructed and evaluated for their impact on motility and adherence in a P30 background. In addition, other motility mutants will be generated by transposition and identified on the basis of loss of satellite growth. Motility mutants retaining the ability to cytoadhere will be characterized further. The genes insertionally inactivated will be identified by sequencing and comparison to the genome sequence. Excision revertants will be isolated, and the motility phenotype will be rescued by complementation with the recombinant wild-type gene by transposon delivery. The proteins associated with gliding motility will be characterized in detail, including determination of subcellular localization. Chemokinesis will be assessed by using a Boyden chamber or compartmentalized petri plates. Cell morphology will be determined by scanning electron microscopy.

**Grant:** 1R01AI049214-01A1  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** NEYFAKH, ALEX A PHD  
**Title:** Antibiotic Hypersusceptibility Mutations in Bacteria  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 2002/02/15-2006/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): The escalating problem of bacterial resistance to antibiotics calls for radical changes in the existing antibacterial therapies. One of the most promising approaches is the use of antibiotic potentiators, compounds that make bacterial cells hypersusceptible to antibiotics. The goal of the project is to identify multiple novel molecular targets for potentiators. This will be accomplished by isolating antibiotic hypersusceptibility mutations of Gram-negative bacteria, *Acinetobacter* and/or *Escherichia coli*. These mutations will specify bacterial proteins whose inhibition is likely to potentiate antimicrobial action of antibiotics. Antibiotic hypersusceptibility is a very difficult phenotype to select, and only few such mutations are known. We have designed and tested a novel genetic strategy for selection of hypersusceptibility mutations, termed SDR. Application of this strategy will identify multiple mutations increasing bacterial susceptibility to beta-lactams (ampicillin, ceftazidime, imipenem), translational inhibitors (erythromycin, linezolid, tetracycline, and chloramphenicol) and fluoroquinolone antibiotics (ciprofloxacin). The molecular mechanisms underlying the effects of the most interesting of these mutations will be analyzed. In addition to identifying promising targets for potentiators, the project will help unravel new aspects of the mechanism of action of antibiotics and new features of bacterial physiology.



**Grant:** 1R01AI049293-01A2  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** RAMAMOORTHY, RAMESH PHD  
**Title:** RpoS and gene expression in *Borrelia burgdorferi*  
**Institution:** TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA  
**Project Period:** 2002/05/01-2007/04/30

DESCRIPTION (provided by applicant): *Borrelia burgdorferi*, the etiologic agent of Lyme disease, cycles in nature between two evolutionarily diverse hosts, an invertebrate tick vector and a vertebrate host. The proliferation and maintenance of the spirochete in the two hosts is guided by several adaptive strategies involving the shuffling of proteins in response to changing environmental conditions. In this respect, molecular changes in the spirochete associated with tick feeding is of particular significance as they precede vertebrate infection. Tick feeding results in a dramatic burst in the spirochetal population, with accompanying changes in protein composition. These changes can be duplicated in vitro by shifting a spirochetal culture to a higher temperature and lower pH environment. One of the key proteins induced under these conditions is the transcription factor RpoS. Our long-term goal is to investigate the molecular events triggered in *B. burgdorferi* in response to changes in environmental temperature and pH. Our short-term objective is to exploit the in vitro model to develop a better understanding of this phenomenon. Specifically, the goals of this proposal are to (1) investigate the mechanism of regulation of RpoS, (2) identify chromosomal RpoS-regulated genes, (3) characterize the role of RpoS in the regulation of the chromosomal RpoS-regulated genes in vivo. The regulation of RpoS expression will be examined, as we and others have determined that its own expression is responsive to changes in temperature and pH. We have identified a putative DNA-binding protein by electromobility shift assay that specifically binds to the *rpoS* 5' sequence. Initially, a response regulator protein RRP-2 will be examined as a putative candidate, failing which the unknown protein will be screened for and identified. The role of this DNA-binding protein in the expression of RpoS will be defined in vitro. We will also identify new chromosomal RpoS-regulated proteins by an in vitro screening assay. This assay will involve in vitro transcription using recombinant *B. burgdorferi* RNA polymerases loaded with sigma D or sigma S. Finally, the regulation of expression of these newly identified genes by RpoS will be partially characterized in vivo. These studies will shed light on the mechanism guiding the flow of genetic information, and identify new effector proteins, in Lyme disease spirochetes poised to infect the vertebrate host.

**Grant:** 1R01AI049294-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** SANDKVIST, MARIA B PHD  
**Title:** Organization and Function of a Type II Secretion Complex  
**Institution:** AMERICAN NATIONAL RED CROSS ROCKVILLE, MD  
**Project Period:** 2002/03/01-2007/02/28

DESCRIPTION (provided by applicant): The type II secretion pathway is widely distributed among Gram-negative pathogens where it is responsible for extracellular secretion of toxins and degradative enzymes. At least 12 gene products located in both the cytoplasmic and outer membrane collectively comprise the type II secretion apparatus, which is specifically required for the translocation of secreted proteins from the periplasm to the extracellular milieu. This pathway is highly specific. It distinguishes secreted proteins from resident periplasmic proteins and discriminates between its own secreted proteins and those introduced from closely related species. In addition, secretion through this pathway differs from most other membrane transport systems in that its substrates consist of folded proteins. The objective of this proposal is to characterize the mechanism of type II secretion at the molecular level. The studies will utilize *Vibrio cholerae* and will specifically examine the secretion of cholera toxin (CT), as well as other proteins that utilize the type II pathway. CT provides an excellent model protein for these studies since its structure and function as well as its folding and assembly pathway are very well characterized. Specifically, we will test the hypotheses that: 1) Molecules secreted by the type II system encode information critical to their secretion within their tertiary structure, 2) the type II complex spans both membranes and components involved in connecting the outer membrane secretion pore with the cytoplasmic membrane regulate secretion by transducing energy to the secretion pore or by regulating its opening, 3) the secretion apparatus is localized to the poles or septum where the peptidoglycan undergoes rearrangements and/or where the pore size is atypical to allow for secretion of folded proteins.

**Grant:** 1R01AI049311-01A1  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** FEY, PAUL D PHD  
**Title:** ica phase variation in Staphylococcus epidermidis  
**Institution:** UNIVERSITY OF NEBRASKA MEDICAL OMAHA, NE  
CENTER  
**Project Period:** 2002/07/01-2006/06/30

DESCRIPTION (provided by applicant): Staphylococcus epidermidis is the preeminent cause of infections involving biomaterial-based devices. The most important step in the pathogenesis of S. epidermidis mediated foreign body infections is the ability of the organism to adhere and to produce biofilm on the surface of the biomaterial. After initial adherence, certain strains of S. epidermidis produce an extracellular biofilm, or polysaccharide intracellular adhesin (PIA), that is encoded by a four gene (icaA, icaD, icaB, and icaC) operon ica. Strains of S. epidermidis deficient in the production of PIA are significantly less adherent in in vitro studies and are less virulent in in vivo models of biomaterial based infections. Preliminary data suggest that PTA production is governed by multiple layers of gene regulation. The ica operon has been shown to undergo a phenomenon termed phase variation, whereby a certain proportion of the population do not produce PTA (biofilm-negative). Phase variation has been hypothesized to have pathogenic significance in that those cells not producing biofilm are less adherent and may be free to disperse and colonize other fertile areas resulting in metastatic disease. Data presented in this proposal demonstrate that there are at least three classes of phase variants. Class I variants can be termed true "phase variants" as they readily revert to wild-type (biofilm forming). These variants do not produce detectable ica transcript yet have an intact ica operon. Class II variants produce little biofilm presumably due to mutations within ica. Class III variants do not produce biofilm due to the loss of a large genomic region which includes ica. The major goal of this study is to elucidate the genetic regulation of PIA production and better characterize the importance of phase variation in the pathogenesis of prosthetic device infection. This proposal has four specific aims: Specific aim 1: Genetic loci that are responsible for regulation of ica transcription, and their relationship to the mechanism governing loss of ica transcription in class I phase variants, will be defined. Specific aim 2: Mutational events responsible for class II phase variants will be characterized and it will be determined whether these mutational events are random or whether they preferentially occur in an ordered manner. Specific aim 3: Class III phase variants will be studied in order to ascertain whether the excision site is conserved amongst different strains of S. epidermidis. Specific aim 4: A guinea pig tissue cage model and mouse foreign body infection model will be utilized to ascertain the pathogenic significance of phase variants. This proposal will yield significant new information regarding fundamental questions of S. epidermidis pathogenesis and biology. Novel means to prevent and treat biomaterial-based infections may be suggested as a result of these studies.

**Grant:** 1R01AI049346-01A2  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** YUK, MING H PHD  
**Title:** Type III Secretion in Bordetella Pathogenesis  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 2002/07/01-2007/06/30

DESCRIPTION (provided by applicant): The type III secretion system of *Bordetella bronchiseptica* is chosen as a model system for studying bacterial-host interactions at the molecular level. *B. bronchiseptica* is a specialized respiratory pathogen closely related to *B. pertussis* (which causes whooping cough in humans). However, *B. bronchiseptica* naturally infects many laboratory animals, which allow us to study bacterial-host interactions in the context of natural infections. We have discovered a type III secretion system in *B. bronchiseptica*. Type III secretion systems are found in several pathogenic Gram-negative bacteria and can deliver proteins directly into the host cytoplasm or plasma membrane upon contact of the bacteria with the host cell. We have identified 22 linked genes in the *B. bronchiseptica* genome encoding proteins for a type III secretion system. By comparing the in vitro phenotypes of the wild type bacteria with a *bscN* deletion strain (that is defective in type III secretion) in their interactions with cultured cell lines, we deduced that type III secreted factors have a variety of effects on host cells. These include: induction of cytotoxicity, inhibition of activation of the transcription factor NF- $\kappa$ B by the aberrant aggregation of this factor in the cytoplasm, and activation of the ERK MAP kinase pathway. In vivo studies showed that type III secretion is required for persistent colonization in the trachea of rats and mice and the down-modulation of anti-*Bordetella* antibody production. Based on these observations, we hypothesize that type III secreted factors from *B. bronchiseptica* play immuno-modulatory roles for the bacteria to attain persistent, chronic colonization. The objective of this study is to identify the specific effector proteins secreted by the type III secretion system in *B. bronchiseptica* and determine how they alter host cellular processes and immune responses. We shall identify type III secreted proteins from *B. bronchiseptica* by both biochemical and genomic approaches, and use in vitro infection models to determine the mechanisms by which the effector protein(s) inhibit NF- $\kappa$ B activation and activate the MAP kinase pathways. We shall also use the mouse infection model to determine the importance of down-regulation of humoral immunity in allowing persistent colonization of the bacteria and how type III secreted virulence proteins interact with host immune cell functions. By identifying the bacterial effector proteins and examining how they interact with host cells, we shall determine the molecular basis for pathogenesis and down-regulation of immune processes.

**Grant:** 1R01AI049352-01A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** ALLAND, DAVID E MD CLINICAL MEDICAL  
SCIENCES, OTHER  
**Title:** TUBERCULOSIS GENOTYPING AND EVOLUTIONARY CONSORTIUM  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2002/09/15-2006/06/30

DESCRIPTION (provided by applicant): We will develop the basic tools for phylogenetic studies of *Mycobacterium tuberculosis* (Mtb), and then investigate hypotheses fundamental to understanding the evolution and strain differentiation of the Mtb species. These issues are important for understanding the biology and epidemiology of many bacterial pathogens. A microbial "species" often encompasses related strains or clones with distinct genotypic and phenotypic characteristics. Studies of epidemiology, pathogenesis, and immunity may depend on the ability to identify and classify strains into larger related groups. Mtb has been recalcitrant to this type of analysis because of its relatively low level of genetic polymorphism. We identified a large number of single nucleotide polymorphisms (SNPs) in Mtb based on a comparison of two complete Mtb genome sequences. Preliminary investigations demonstrated that these SNPs are highly polymorphic in clinical Mtb strains and are excellent phylogenetic markers. We then sequenced (to 5X coverage) the genome of a third clinical Mtb strain. Preliminary analysis confirms that comparisons with this strain provide an additional set of novel SNP markers. We also developed a new method for high-throughput SNP analysis that is simple and inexpensive. We now propose to develop a SNP-based phylogenetic model of the Mtb species, and to investigate basic hypotheses about Mtb phylogenetics, evolution, and epidemiology. Our aims are 1) To determine the minimum number and type of SNP markers required to define a high-resolution phylogenetic tree of the Mtb species, and to construct such a tree with a minimal SNP set. This aim will discover novel and informative SNPs by performing a six-way comparison between the genomes of H3 7Rv, CDC1551, the 210 strain, and *M. bovis*. 2) To investigate basic phylogenetic hypotheses including whether the SNP-based phylogenetic data is tree-like, clock-like, and reproducible. We will also investigate if the Mtb species contains a common origin and undergoes introgression or lateral gene exchange; and we will investigate the evolutionary relationships between different Mtb strains and *M. bovis*. 3) To use the SNP-based phylogeny to investigate other analytic systems including IS6110 and VNTR-based DNA fingerprinting; and to provide insights into TB molecular epidemiology and evolution. 4) To identify and validate an informative set of SNPs for worldwide evolutionary and epidemiological analyses.

**Grant:** 1R01AI049354-01A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** RAMSEY, KYLE H PHD  
**Title:** Host Factors in Susceptibility to Chlamydial Disease  
**Institution:** MIDWESTERN UNIVERSITY DOWNERS GROVE, IL  
**Project Period:** 2002/03/01-2007/02/28

DESCRIPTION (provided by the applicant): Chlamydia trachomatis infections are the most commonly reported transmissible diseases in the U.S. Diagnosis, treatment, and sequelae of chlamydial disease cost billions of dollars each year in the U.S. alone. The infection is often asymptomatic in women. Variations in the host immune response are likely to blame for adverse outcomes because not all persons who become infected will suffer the long-term consequences of the disease. In those who progress to disease, the affected tissues are significantly altered in their structure and function by a process that ultimately results in scarring and blockage of the fallopian tubes. This results in tubal factor infertility and risk of ectopic pregnancy. Our hypothesis is that those who sustain this outcome have dysregulation of factors which are responsible for the repair of the extracellular matrix. To address hypothesis, we will use a mouse model of chlamydial disease where inbred strains exist which have been characterized as resistant or susceptible as indicated by the outcomes of tubal damage and infertility. In approach, we will first extensively compare and contrast these strains with regard to their ability to modify and repair the extracellular matrix of the urogenital tract in vivo and in vitro. Subsequently, we will define the role of matrix metalloproteinases (MMPs) in the outcome of chlamydial disease through in vivo studies where the enzymes are inhibited pharmacologically or cytokines that influence their activity and production are neutralized. We will then define a role of specific metalloproteinases to the disease process through the use of mice with deletions in genes that encode the enzymes. Lastly, the contribution of specific inflammatory cells to the modulation of extracellular matrix in chlamydial disease will be defined by the production of bone marrow chimeras between susceptible and resistant strains of mice and subsequent depletions of leukocyte populations. In summary, it is the intent of this proposal to define host factors that are responsible for adverse chlamydial disease outcome. The information derived will assist in the development of therapies which could ameliorate the chlamydial disease process; noninvasive diagnostic indicators of progressive scarring and abnormal physiological outcome; development of prognostic indicators of those at high risk for chlamydial disease; and, further advances in design of a safe and effective chlamydial vaccine through avoidance of adverse outcomes.

**Grant:** 1R01AI049388-01A2  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** WETZLER, LEE M  
**Title:** Effect of Neisserial Porin in Immune Cell Apoptosis  
**Institution:** BOSTON MEDICAL CENTER BOSTON, MA  
**Project Period:** 2002/07/01-2007/06/30

Neisserial porins (gonococcal Protein I [PIA or PIB] or meningococcal class 1 [PorA], 2 or 3 [PorB] proteins) are the major protein constituents of the Neisserial outer membrane. We have previously demonstrated that the Neisserial porins activate B lymphocytes, increasing the surface expression of the costimulatory ligand B7-2, which improves the B cell's ability to "costimulate" T lymphocytes. The induction of B7-2 expression is directly related to the porins' adjuvant ability. During the course of this work, we have discovered that the porins, when activating B cells, decreased the susceptibility of these B cells to activation induced cell death (AICD) or apoptosis. We found that the anti-apoptotic effect is due to a direct interaction of the porin with mitochondria (a central control point for apoptotic cell death). Immunoprecipitation experiments revealed that PorB interacts with the mitochondrial porin, VDAC. We hypothesize that this VDAC-Neisserial porin protein-protein interaction, which is similar to that of VDAC with the anti-apoptotic molecule Bcl-2, is responsible for the anti-apoptotic effect of the porins. This interaction results in an enhancement of cell survival and continued activation of B cells, which would potentiate the B cells' involvement in specific immune responses. Therefore, it is possible that this anti-apoptotic effect is a potential additional mechanism of the porins' immunopotentiating ability. This proposal will investigate the effect of Neisserial porins on the susceptibility of B cells, epithelial cells and other cell types to apoptosis. The basic aims include 1) examining the interaction of Neisserial porin with mitochondria and mitochondrial VDAC, 2) determining if alterations in cell culture conditions (especially serum withdrawal) can alter the effect of porins on apoptosis and whether this could be related to changes in cellular ATP levels and 3) determining the effect of intact Neisserial organisms on apoptosis and whether porin might play a role in this effect. These studies will yield significant findings that could both elucidate the immune stimulatory effect of Por (and potentially other bacterial components), allowing for the better use of Por as a vaccine adjuvant. In addition, these results can have significant implications in Neisserial pathogenesis, especially since we have new preliminary data that intact live meningococci can also protect cells from apoptosis.

**Grant:** 1R01AI049424-01A1  
**Program Director:** AULTMAN, KATHRYN S.  
**Principal Investigator:** MUNDERLOH, ULRIKE G PHD ENTOMOLOGY AND  
PARASITOLOGY  
**Title:** Development of Paratransgenic Ticks for Disease Control  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2002/07/01-2007/04/30

**DESCRIPTION:** (provided by the applicant): Tick-borne diseases are increasingly diagnosed in humans and animals. Some are due to the resurgence of previously known illnesses, like Rocky Mountain spotted fever (Walker 1995). but others are due to new, emerging pathogens. Among the spotted fever group (SFG) alone, 8 new human pathogens have been described in the last 15 years (Stenos et al. 1997; Nilsson et al. 1999; Fournier et al. 2000), but they also include viruses, ehrlichias and Babesia (Dawson et al. 1991; Thomford et al. 1994; Bakken et al. 1994; Telford et al 1991). Novel, efficient, specific and environmentally acceptable methods that interfere with disease transmission by ticks are urgently needed. Using paratransgenic ticks that carry symbiotic prokaryotes expressing an antimicrobial substance, as has been achieved with the symbiote of the Chagas disease vector, *Rhodnius prolixus* (Durvasula et al 1997), could offer a safe and effective way to reduce disease transmission by ticks. A major obstacle to accomplishing this goal has been the lack of culture systems for tick symbiotes. Our laboratory has the largest collection of tick cell lines. We have successfully used these to isolate tick-associated rickettsiae (Munderloh et al. 1998; Weller et al. 1998; Palmer et al. 1999; Simser et al. 2001a,b) from the Lone Star tick (MOAa), the Rocky Mountain wood tick (*R. peacockli* DAE100R), and the Castor Bean tick (*Rmoreli* T2). We have characterized these microbes by light and electron microscopy, by using specific antibodies, as well as by PCR and nucleotide sequence analysis of 16S rDNA and other key genes. We are now in the process of defining the cultures to facilitate genetic manipulation of the symbiotes. Our long-term aim is the stable transformation of *Rickettsia peacockii* with *cecropinA*, an insect toxin gene (Hultmark et al. 1983). Infection of ticks with the transformed rickettsia, and interference with pathogen transmission. We plan to target the non-functional *rompA* gene of *R. peacockli* as a site for homologous transformation, avoiding deleterious effects associated with disruption of a vital gene, e.g. the *rpoB* gene (Troyer et al. 1999). We will take the recent advances in successful transformation of insect-borne rickettsiae as a guide (Rachek et al. 1998; Troyer et al. 1999). and also apply transposome technology (Epicentre). Specifically, we will 1. optimize culture conditions for production of *R. peacockii* in tick cell culture, examine its behavior in tick and mammalian cell culture by light and electron microscopy. 2. We will analyze cultured *R. peacockii* in ticks in terms of tissue tropism and transstadial/transovarial passage, and sensitivity to *Cecropin A*. Finally, we will work towards 3. stable transformation of *R. peacockii* with *cecropinA*. We will then test the transformants for antimicrobial activity in vitro and in ticks, and characterize them by sequence analysis.



**Grant:** 1R01AI049437-01A2  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** AKERLEY, BRIAN J  
**Title:** Global regulatory interactions in bacterial pathogenesis  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/06/01-2007/05/31

DESCRIPTION (provided by applicant): *Haemophilus influenzae* efficiently and chronically colonizes the human nasopharyngeal mucosa, and is capable of causing invasive disease including otitis media, pneumonia, and, more rarely, meningitis. A number of factors involved in *H. influenzae* virulence have been identified in the pre-genomic era. Taking advantage of the genome sequence and the advent of new technologies, such as global expression profiling, we intend to advance understanding of critical virulence characteristics of this organism. Lipopolysaccharide (LPS) structural modifications are essential virulence determinants for *H. influenzae*. Using expression profiling with DNA microarrays, complemented by classical approaches, we have recently uncovered a previously unappreciated link between redox regulation and LPS modifications in *H. influenzae*. In addition, we have isolated a mariner transposon insertion mutation in *H. influenzae* that disrupts redox control over one such modification (addition of a phosphorylcholine epitope, termed ChoP, to the LPS) and also results in a pronounced colonization defect in an animal model of *H. influenzae* infection. These observations are of potential significance for in vivo modulation of the LPS structure by environmental signals. We propose to use such signaling and regulatory mutants generated in our laboratory to examine the role of redox signaling in controlling virulence genes in *H. influenzae*. Global genomic approaches we have developed for studies of *H. influenzae* will facilitate our analysis of how LPS modifications are modulated in response to environmental conditions. We will also determine whether other genes that play a role in pathogenesis are coregulated, inversely regulated, or constitutively transcribed under the varied redox conditions that affect LPS modification. We believe that these studies will provide important insights into the relationship between physiological adaptations to the host environment and the coordinated production of bacterial cell-surface structures critical for interactions with host cells or for evading the immune response. Specifically, we will: 1. Characterize the redox control mechanisms involved in the regulation of the ChoP cell surface LPS modification. 2. Investigate the role of signaling pathways in *H. influenzae* in the context of epithelial cell interactions and in a model of respiratory tract infection. 3. Examine coordinate regulation of virulence factors by redox signaling systems.

**Grant:** 1R01AI049534-01A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** JAGANNATH, CHINNASWAMY  
**Title:** Characterization of an M. tuberculosis vaccine  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX  
HOUSTON  
**Project Period:** 2002/01/01-2006/12/31

Tuberculosis is resurgent in most of the world fueled by drug resistance, AIDS, poverty and mobility. The goal of this project is to produce an improved vaccine. It is based on our observations that an antigen 85A deletion mutant of MTB strain H37Rv (Ag85A-) is markedly attenuated and has increased antigen presenting capability. Our rationale has three components: First, the most effective immunity known against tuberculosis is provided by prior infection with MTB itself. Consequently, we propose that an appropriately attenuated MTB will approach the natural limit of efficacy of vaccines for tuberculosis. Second, MTB inhibits phagosome-lysosome (P-L) fusion. Preliminary data demonstrates that deletion of the Ag85A gene of MTB restores P-L fusion, enhances antigen presentation and probably increases the immunogenicity of MTB. Third, since MTB is a clonal organism with no demonstrated ability for horizontal transfer of genes, the safety of attenuated MTB can be assured. The specific aims are: 1) Evaluate the efficacy and safety of the Ag85A-mutant as a vaccine against tuberculosis. The efficacy (ability to limit primary infection and dissemination) will be investigated by single or multiple immunizations of C57BL/6 mice, outbred mice and guinea pigs prior to aerosol challenge with virulent MTB. Safety will be evaluated by infection of a spectrum of animals including guinea pigs that are naturally more susceptible to disease, genetically heterogeneous outbred mice, immunocompromized SCID mice and steroid treated mice. Safety will be evaluated in terms the capacity of the vaccine organism to produce disease and its effect on persistence and pathogenicity of wild type MTB following challenge. 2) Introduce additional deletion mutations into the Ag85A- strain to improve safety while maintaining immunogenicity. We anticipate that any vaccine with prolonged survival in tissue may produce disease in immunosuppressed people. Consequently, attempts will be made to produce a double knockout mutant MTB that retains the immunogenicity of Ag85A- and is unable to survive in tissue. The 16 kDa alpha crystallin protein gene and nitrate reductase gene will be targeted. We anticipate that a safe live attenuated MTB vaccine with enhanced immunogenicity will prove valuable in combating adult pulmonary tuberculosis as well primary disease.

**Grant:** 1R01AI049978-01A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** ZHOU, DAOGUO PHD  
**Title:** Actin-Cytoskeleton Rearrangements by Salmonella  
**Institution:** PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN  
**Project Period:** 2002/04/01-2007/03/31

Despite improvements in public hygiene, salmonellosis continues to cost the world economy billions of dollars each year and remains to be the number one cause of reported foodborne diseases. The Salmonella infection involves complex and highly orchestrated interactions between the bacterium and host cells. Salmonella injects proteins into host cells via a bacterial type III secretion system. Our working hypothesis is that these bacterial proteins engage host proteins for actin polymerization as well as depolymerization, two processes that are required for Salmonella-induced actin cytoskeleton rearrangements and invasion of non-phagocytic cells by the bacterium. The goal of this project is to identify and characterize bacterial and host proteins that play a role(s) in modulating actin dynamics both in vitro and in vivo by using microbiological, biochemical and cellular approaches. This proposal focuses on the molecular mechanism of Salmonella-induced actin rearrangements involving SipA. We have shown that SipA binds actin and modulates actin dynamics by decreasing the critical concentration for actin polymerization and by inhibiting depolymerization of actin filaments. We also showed that SipA increases the bundling activity of T-plastin, which increases the stability of actin bundles. Preliminary results indicate that additional host proteins are present in the SipA-actin complex and SipA activities must be turned off by other bacterial or host factors. We propose to investigate how Salmonella-induced actin cytoskeleton rearrangements are initiated, maintained and subsequently reversed. We have developed assays and reagents necessary to examine the actin architecture and investigate roles of SipA and host proteins in modulating Salmonella-induced actin cytoskeleton rearrangements. Results from this study will help us understand how Salmonella intercepts normal cellular constituents to modulate host actin cytoskeleton. A better understanding of these processes will facilitate the development of new chemotherapeutic agents for the treatment and prevention of salmonellosis. These experiments will also provide new insights into basic host cellular functions, including cytoskeletal rearrangements and cell movement.

**Grant:** 1R01AI049987-01A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** DIETRICH, WILLIAM F  
**Title:** Molecular Pathways of Host Susceptibility to Legionella  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 2002/02/15-2007/01/31

Legionella pneumophila is a significant human lung pathogen that is responsible for many community- and hospital- acquired cases of pneumonia. An important part of the pathogenesis of Legionella infection is its ability to grow inside of macrophages. Interestingly, different inbred strains of mice exhibit differences in the permissiveness of their macrophages for the intracellular replication of Legionella, and these differences have been attributed to genetic differences in one of the mouse Neuronal Apoptosis Inhibitory Protein (Naip) genes. The goals of this project are to: Establish the identity of the Naip gene and its mutations that are responsible for the differences in Legionella permissiveness, and to study the function of the Naip proteins by identifying critical interacting proteins that participate in its molecular function.

**Grant:** 1R01AI050000-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** OTTEMANN, KAREN M BS  
**Title:** Roles for Motility in Helicobacter pylori pathogenesis  
**Institution:** UNIVERSITY OF CALIFORNIA SANTA CRUZ SANTA CRUZ, CA  
**Project Period:** 2002/04/01-2007/03/31

The bacterium *Helicobacter pylori* inhabits the stomachs of a full 3 billion people--half the world's population. Infections with this bacterium cause significant morbidity and mortality around the globe. In the majority of cases, the bacterium establishes chronic infections that lead to diverse outcomes ranging from asymptomatic carriage to ulcer disease to gastric cancer. Conservative estimates suggest that 5 percent of those infected--150 million people--develop some form of disease. *H. pylori* has the dubious distinction of being the only bacterium classified as a Group I carcinogen by the International Agency for Research on Cancer of the World Health Organization. Infection by this bacterium is a risk factor for several types of gastric cancer including gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The processes used by *H. pylori* to establish and maintain infection are just beginning to be worked out. One such process is the ability to swim. *H. pylori* is motile via organelles called flagella, and it must have functional versions of these organelles in order to colonize animal stomachs. In addition, *H. pylori* does not swim randomly but instead directs its motility in response to environmental cues in a process called chemotaxis. We are interested in understanding how chemotactic motility is used for infection by this bacterium. Towards this we propose three aims: (i) Ascertain at what points during infection chemotactic motility is used by *H. pylori*. (ii) Determine how *H. pylori* chemotaxis is directed by pinpointing which of its chemoreceptors are used for mouse stomach colonization, and to what they respond. (iii) Dissect how information is relayed from the chemoreceptors to the flagellar motor during chemotactic signal transduction in *H. pylori* by analyzing how a family of proteins, the CheVs, function. Chemotaxis is observed in a number of bacterial pathogens but little is known about its role during infection. What we learn from these studies may apply to other bacteria. For example, the *Campylobacter* group of bacteria are leading causes of food-borne diarrheal disease, and similarly require motility and chemotaxis for infection.

**Grant:** 1R01AI050027-01A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** ADAMS, LINDA B PHD  
**Title:** GENE KNOCK-OUT MICE AS MODELS FOR THE LEPROSY SPECTRUM  
**Institution:** NATIONAL HANSEN'S DISEASE PROGRAM BATON ROUGE, LA  
**Project Period:** 2002/05/01-2007/04/30

DESCRIPTION (provided by the applicant): The proposed studies will explore Mycobacterium leprae foot pad infection in knockout (KO) mouse strains carefully selected for their disruption in genes that play key roles in host cell mediated immunity (CMI) to mycobacterial pathogens. Growth of M. leprae in the foot pad will be monitored and the experimental granulomas which develop will be analyzed to determine if these KO mouse strains can serve as models for the key immunoregulatory elements of CMI that result in the unique immunopathological spectrum of human leprosy. CMI responses will be further modified in the KO mice by conditionally knocking-out additional gene products before or after infection with M. leprae or by selectively restoring certain disrupted gene functions after infection. Development of KO mouse models for discrete elements of the human leprosy spectrum should open investigation into the mechanisms underlying the instability inherent to the borderline area of this spectrum where downgrading and upgrading shifts toward the lepromatous and tuberculoid ends of the spectrum, respectively, are poorly understood. More importantly, KO mouse models of leprosy and the additional manipulations of these models that are proposed may afford insight into the mechanisms responsible for the abrupt onset of type 1 and type 2 reactions. Ultimately, this basic knowledge may permit prediction and prevention of these devastating reactions, which markedly enhance nerve damage. Numerous studies have been reported with M. tuberculosis in gene KO mice. We suggest that M. leprae-KO mouse studies will permit more detailed dissection of the mechanisms of CMI. Targeted removal of a number of isolated gene functions often greatly exacerbates experimental murine tuberculosis, perhaps by overwhelming certain compensatory mechanisms in host resistance. In marked contrast, M. leprae is a quiet, well adapted, obligate intracellular pathogen. This proposal is based on the likelihood that its characteristics of slow rate of growth, low virulence and chronic pathogenesis are the very attributes which will make the study of M. leprae in targeted gene KO mice an ideal model for analyzing the principal redundant and compensatory mechanisms of CMI in host resistance to infection in general and to intracellular mycobacterial pathogens in particular.

**Grant:** 1R01AI050032-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** WATNICK, PAULA I MD  
**Title:** Biofilm Development by *Vibrio cholerae*  
**Institution:** NEW ENGLAND MEDICAL CENTER BOSTON, MA  
HOSPITALS  
**Project Period:** 2002/05/01-2007/04/30

DESCRIPTION (provided by the applicant): *Vibrio cholerae* is the causative agent of cholera, a disease characterized by severe diarrhea and dehydration. As a natural inhabitant of oceans, estuaries, rivers, and lakes, *V. cholerae* is well adapted for survival in both fresh water and marine environments. *V. cholerae* biofilms have been observed in the human intestine and in the aquatic environment. The goal of this research is to identify and characterize the genes and environmental signals that guide bio film development by *V. cholerae* in diverse aquatic environments. Results of this work may provide a scientific basis for the correlation of epidemic cholera with environmental parameters, increase our understanding of the diversity of biofilms formed by gram-negative organisms, and form the basis for rational design of inhibitors of bacterial attachment to be used in environmental approaches to the control of cholera and in the prevention of biofilm-associated infections including central venous catheter sepsis, prosthetic joint infections, and endocarditis. The Specific Aims of this research are: 1) To study the mechanism of action of Bfr 1, a recently identified, novel regulator of biofilm development, 2) To identify and characterize additional environmental signals and regulators that guide *V. cholerae* biofilm development in fresh water, and 3) To identify and characterize the environmental signals and regulators that guide *V. cholerae* biofilm development in sea water.

**Grant:** 1R01AI050086-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** PETERSON, JOHNNY W PHD  
MICROBIOLOGY:MICROBIO  
OGY-UNSPEC  
**Title:** New Mechanisms in the Pathogenesis of Cholera  
**Institution:** UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX  
GALVESTON  
**Project Period:** 2002/05/01-2007/04/30

DESCRIPTION (provided by applicant): The objective of our proposal is to define the mechanism of action of cholera toxin (CT), and our hypothesis is that arachidonic acid (AA) metabolites (e.g., prostaglandin E2 [PGE2] and leukotriene C4 [LTC4] exert a significant impact on the physiological functions of the small intestine. While CT can directly upregulate adenylate cyclase activity by catalyzing the ADP-ribosylation of Gs-alpha and increase 3, 5 adenosine monophosphate (cAMP) levels, equally important are stimulatory effects on AA metabolism leading to increased production of eicosanoids (e.g, PGE2 and LTC4). PGE2 can stimulate adenylate cyclase and intestinal ion transport, while LTC4 is known to stimulate Ca++ mobilization. Further, Ca++ channel blockers reduce CT-induced fluid transport. Importantly, our Preliminary Studies show that CT stimulates AA metabolism independent of ADP-ribosylation of Gs-alpha by signaling the expression of the plaa gene, which encodes phospholipase A2-activating protein (PLAA). Immunoelectron microscopy and plaa antisense oligonucleotide experiments show that PLAA is an important nucleoprotein that upregulates PLA2 activity. After cloning human plaa cDNA, we overexpressed the gene in several prokaryotic and eucaryotic systems, We have also observed the celecoxib, a highly specific COX-2 inhibitor, blocks synthesis of PGE2 and CT-induced fluid transport in murine intestinal segments. We discovered novel imidazole covalent analogs of PGE2 that reduce cAMP levels and fluid accumulation in murine intestinal loops challenged with CT. In Aim1, intestinal cells (e.g., Paneth, epiteilial) that synthesize PLAA in response to CT will be identified using in situ hybridization and immunohistochemistry, and the role of PLAA and Paneth cells will be established with PLAA and Paneth cell knockout mice. The role of the major constituents of the AA pathway (e.g., PLA2, COX-2, PGE2 synthase [PGES], and PGE2 receptors) will be evaluated in Aim 2 using specific inhibitors and knockout mice. In Aim 3, we propose to define the regulatory mechanism by which CT signals the transcription of the plaa gene and PLAA synthesis. Aim 4 consists of using plaa cDNA expressed in eucaryotic cells and PLAA protein to evaluate stimulatory effects on PLA2 activity. Although CTs action is a multifaceted mechanism, therapeutic control of CT-induced hypersecretion of water and electrolytes in cholera may be possible by modulating AA metabolism.



**Grant:** 1R01AI050122-01A1  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** HECHT, DAVID W MD OTHER AREAS  
**Title:** Efficient Mechanism of Gene Transfer in *B. fragilis*  
**Institution:** LOYOLA UNIVERSITY CHICAGO MAYWOOD, IL  
**Project Period:** 2002/03/15-2007/02/28

The hypothesis of this study is that DNA and antibiotic resistance gene transfer in *Bacteroides* spp is highly efficient, involving genetically unrelated mobilization modules (one to three genes) that perform all DNA processing functions during transfer initiation. Further, these mobilization modules are located on different transfer factors. They all interact similarly with a conjugation apparatus (mating pore) that is encoded by *Bacteroides* spp Tet elements, resulting in their widespread dissemination. The rationale for this hypothesis is derived from extensive preliminary data demonstrating that *Bacteroides* transfer factors have similar transfer characteristics in *Bacteroides*, when co- resident with a common mating apparatus, and to *E. coli* when co- resident with a broad host range plasmid. Each of the mobilizable transfer factors contains one, two, or three genes that must perform similar DNA processing functions, despite a lack of homology. However, all mobilizable transfer factors have a five bp consensus *nic* sequence. We have now captured a *Bacteroides* mating apparatus, which appears to be part of a conjugative transposable element (Tet element). This mating apparatus provides the mating bridge for multiple mobilization factors in *Bacteroides*, and also functions in *E. coli*. Further, both Tn5520 and the Tet element are widely disseminated in *Bacteroides*. Thus, *Bacteroides* may represent a highly efficient model for DNA and antibiotic resistance dissemination. The Specific Aims of this study are to 1) determine that all DNA processing functions of the promiscuous Tn5520 transfer factor are provided by a single mobilization protein and *oriT* region, and 2) to determine that pLF9 encodes a mating apparatus that serves as a common pathway for many different conjugative transfer factors, and identify key gene(s) involved in the interaction between initiation complexes and the mating pore. Studies include the use of matings, insertion and deletion mutagenesis, in vitro and in vivo relaxosome formation, DNA sequencing, protein/protein interactions, and cellular localization experiments. Upon completion of these studies, we will have determined the minimal requirements for DNA processing involved in transfer from *Bacteroides*, allowing comparison to more complex systems in other bacteria. The identification and characterization of the key components of the mating apparatus and relaxosome complex that allow for utilization of a single mating pore by genetically diverse transfer factors will be a major step towards a comprehensive model of DNA transfer in *Bacteroides*, and will provide important insights as to how bacterial antibiotic resistance is rapidly spread.

**Grant:** 1R01AI050216-01A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** REMOLD, HEINZ G MD  
MULTIDISCIPLINARY: MULTIDISCIPLINARY  
LIN, BASIC MED  
**Title:** A Novel Mechanism of Innate Immunity Against TB  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2002/04/01-2007/03/31

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

**Grant:** 1R01AI050217-01A1  
**Program Director:** SAVARESE, BARBARA M.  
**Principal Investigator:** VISCIDI, RAPHAEL P MD  
**Title:** Molecular Epidemiology of N. gonorrhoeae  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/03/01-2007/02/28

Gonorrhea is a common bacterial infection that is transmitted primarily by sexual contact. Gonococcal infections have an epidemiological pattern characterized by the concentration of infections in social or geographically defined communities termed "core groups". These "core groups" have been proposed as reservoirs for the continued transmission of infections within communities, and therefore, understanding the biological nature of the constituent organisms is important for public health control strategies. We propose to examine the molecular evolution of *Neisseria gonorrhea* within a community over time and in relation to epidemiological information pertaining to the host individuals. This is a collaborative effort between two experts in infectious disease and epidemiology of gonorrhea and a population geneticist. Two molecular typing methods will be used to characterize gonococcal isolates: por gene sequencing and multi-locus sequence typing (MLST) scheme, in which alleles at six housekeeping genes are characterized by sequencing approximately 500 bp internal fragments of the genes. The study has three main objectives. The first is to determine temporal trends in the population genetic structure of *N. gonorrhea* over a 20-year period in a high prevalence community, Baltimore, MD. The second is to correlate changes in population genetic parameters with epidemiological information pertaining to the host individual's residence in a "core" or "peripheral" region. Our major hypothesis is that higher levels of genetic diversity, more intense selection pressure on the por gene, a positive growth rate, and a higher recombination rate relative to the peripheral population will characterize the population genetic structure of *N. gonorrhea* in the core. The third aim is to determine whether gender, age, or disseminated versus local infection influences the population genetic structure of *N. gonorrhea*.

**Grant:** 1R01AI050450-01A1  
**Program Director:** LAMBROS, CHRIS  
**Principal Investigator:** CUSHION, MELANIE T  
**Title:** New Approaches for Development of PcP Therapy  
**Institution:** UNIVERSITY OF CINCINNATI CINCINNATI, OH  
**Project Period:** 2002/06/01-2007/05/31

DESCRIPTION (provided by applicant): Pneumocystis carinii (PcP) remains an important cause of infection in immunocompromised hosts including patients with AIDS. The most widely utilized therapy is trimethoprim-sulfamethoxazole (TMP-SMX). Recent concerns have arisen over development of mutations to the DHPS locus, which mediates sensitivity to SMX. Accordingly, Dr. Cushion proposes new strategies to develop treatments for Pneumocystis based upon a better understanding of the sterol and mitochondrial metabolism pathways as potential targets for therapy. They propose to first identify efficacious compounds which target enzymatic steps in the sterol biosynthetic and mitochondrial pathways of Pc using in vitro viability assay. Under the second aim, they will assess the potential mechanisms of action of these inhibitors on each pathway by analysis of gene expression using macroarrays. In the third aim, they will attempt to identify synergistic combinations of the sterol and mitochondrial inhibitors within and between these pathways by construction of inhibitor isobolograms. Finally, they will select the most efficacious combinations of agents and evaluate their effects on gene expression again using the macroarray approach.

**Grant:** 1R01AI050470-01A1  
**Program Director:** LAUGHON, BARBARA E.  
**Principal Investigator:** REYNOLDS, ROBERT C PHD  
**Title:** Inhibitor of FtsZ Polymerization in M. tuberculosis  
**Institution:** SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, AL  
**Project Period:** 2002/06/15-2005/05/31

DESCRIPTION (Provided by the applicant): Development of new antitubercular agents is of critical importance worldwide. Our program has identified a new class of inhibitor of Mycobacterium tuberculosis (Mtb) that inhibits a novel protein not presently targeted by current antitubercular agents. The 2-alkoxy-carbonylamino-pyridines (2-ACPs) potentially inhibit the growth of Mtb with an MIC<sub>99</sub> (SRI-3072) as low as 0.15 microgram/ml (0.28 micromolar). Furthermore, SRI-3072 shows bactericidal activity, and shows significant activity in a murine-derived macrophage model with an EC<sub>90</sub> & EC<sub>99</sub> of 0.12 and 1.42 microgram/ml respectively. These analogs also show selective activity against Mtb versus a mammalian cell line. This program has successfully identified the target of these agents, the mycobacterial tubulin homolog FtsZ. The target protein has been cloned, expressed and isolated in quantities sufficient for development of in vitro polymerization and GTP hydrolysis assays. Three compounds, SRI-3072, SRI-7614, and colchicine have been shown to inhibit polymerization of Mtb FtsZ in a dose dependent manner with IC<sub>50</sub>s of 50 uM, 60 uM, and 100 uM respectively. Furthermore, we have shown that SRI-7614 affects Mtb FtsZ polymerization by electron microscopy. SRI-7614 has also been shown to be active vs. a panel of single drug-resistant Mtb strains. We currently have crystal structures of Mtb FtsZ bound to citrate, GTPγS, and GDP. To date, about 200 2-ACP analogs have been screened in vitro against Mtb H37Rv. We have developed a SAR profile that will allow the preparation of more selective and more potent antitubercular agents. In this application, we propose to continue development of the 2-ACP class through preparations of new analogs of the more potent and selective subclasses, the 3-deaza-pteridines (priority), and the pyridodiazepines (backup). We will carefully evaluate these compounds for activity and selectivity in various in vitro assays including an in vitro Mtb H37Ra assay, an in vitro Mtb FtsZ polymerization and GTPase assay, an in vitro tubulin polymerization assay and a mammalian cell toxicity assay. Selected active agents will be further screened in an in vitro macrophage model and a Mtb mouse model. The effect of inhibitors on FtsZ polymerization will be analyzed using electron microscopy. Data from the biological screening and the EM structural studies will feed back into compound design in an interactive, iterative drug design cycle that critically focuses on antibacterial potency and selectivity.

**Grant:** 1R01AI050661-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** MC FALL-NGAI, MARGARET J PHD  
**Title:** *Vibrio fischeri* as a model of bacterial colonization  
**Institution:** UNIVERSITY OF HAWAII AT MANOA HONOLULU, HI  
**Project Period:** 2001/12/01-2006/11/30

Many animal epithelial tissues are colonized by benign, often essential, bacterial symbionts, such as those in the oral cavity and along the apical surfaces of the cells lining the intestinal and respiratory tracts. These tissues, which occur at the interface between the environment and the body, are also the sites of infection by many bacterial pathogens (e.g., *Haemophilus influenzae* and *Neisseria meningitidis*). In both pathogenic and cooperative associations the bacteria and host cells communicate most commonly through interactions of their surface molecules or through the secretion of other bioactive compounds. The model symbiosis between the epithelial tissues of the squid *Euprymna scolopes* and its Gram-negative, luminous bacterial associate *Vibrio fischeri* is being exploited as a system by which to characterize these cross-Domain cell-cell interactions and to provide insight into the critical differences between beneficial and pathogenic animal-bacterial associations. Recent studies of this system have shown that lipopolysaccharide (LPS), the principal surface molecule of Gram-negative bacteria and the product responsible for the most dramatic mammalian responses to infective bacteria, induces specific changes in the squid host's cell biology and morphology during the initial stages of the relationship. In addition, characterization of host responses to LPS has revealed that this molecule works synergistically with proteinaceous compounds exported by the symbiont. The types of host cell 'behaviors' induced by these *V. fischeri*-derived factors include: the production of mucus to facilitate colonization of susceptible tissues, and the onset of a morphogenetic program that transforms host tissues from a morphology characteristic of the uncolonized juvenile to one characteristic of the mature, adult association. Several other identified responses of host cells to *V. fischeri* may also be affected by these compounds. The goal of this interdisciplinary project is to characterize bioactive molecules of *V. fischeri*, specifically LPS and the peptides that work in concert with LPS, that mediate host tissue responses during the early phases of the squid-vibrio association. The specific aims of this proposal are to: (1) characterize the structures of wild-type *V. fischeri* LPS and the LPS derived directly from the symbiosis, as well as characterize LPS derived from specific LPS-synthesis mutants that are associated with compromised host responses; (2) determine the precise biochemical identity and activity of bacterial molecules that act synergistically with LPS; and, (3) determine what aspects of LPS structure play a role in conferring resistance to host antimicrobial peptides.

**Grant:** 1R01AI050666-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** LUKOMSKI, SLAWOMIR PHD  
**Title:** Sc1 Proteins of S pyogenes: Biology and Function  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2002/05/01-2006/04/30

Group A Streptococcus (GAS) is a major cause of human mortality and morbidity worldwide. Therefore, understanding the molecular mechanisms by which GAS colonizes and invades human hosts is important for the development of new anti-streptococcal therapies. It is clear that cell surface GAS products participate in disease. Two recently identified streptococcal genes, scl1 and scl2, encode cell surface proteins that contain Gly-X-X collagen-like repeats. This last characteristic of Scl is particularly intriguing due to the fact that antibodies against streptococcal cell-wall antigens cross-react with human-tissue antigens, resulting in post-infection autoimmunity. However, nothing is known about Scl protein expression in vivo and antigenicity for the host. The long-term objective of the proposed work is to determine the importance of Scl in GAS adherence to and invasion of human cells and to evaluate the role of anti-SCL response in human autoimmune diseases. This project will test the hypotheses that (a) Scl virulence factors contribute to Gas pathogenesis, (b) expression of scl genes is coordinately regulated by various control mechanisms and many GAS strains simultaneously produce both Scl1 and Scl2 proteins, and (d) Scl proteins are expressed in vivo and anti-Scl-specific antibodies are produced by the infected host. The specific aims are as follows: First, determine whether Scl proteins participate in the pathogenesis of different disease types caused by GAS by constructing genetically defined isogenic scl1 and scl2 mutants of GAS strains and testing them in mouse infection models; second, determine whether Scl proteins contribute to GAS adherence to human cultured cells and extracellular matrix components; third, determine transcriptional and translational mechanisms controlling scl gene expression and whether GAS strains simultaneously produce both Scl proteins; and fourth, analyze expression of Scl proteins within infected tissue and production of anti-Scl-specific antibodies in human sera and in experimental models. The proposed studies will provide new directions for the development of anti-streptococcal therapies and, in light of the existence of the collagen-like antigen Scl on the surface of GAS, may have important implications for future studies of human autoimmune diseases.

**Grant:** 1R01AI050667-01A1  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** HUEBNER, JOHANNES MD  
**Title:** Role of Enterococcal Polysaccharides in Virulence  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2002/08/01-2007/05/31

DESCRIPTION (provided by applicant): The long-term goal of this study is to understand the molecular, biochemical, and immunologic factors that contribute to the pathogenesis of *Enterococcus faecalis* and *Enterococcus faecium* infections. The understanding of the interaction between bacteria and the human host defense system will be the basis for the development of new means of preventing otherwise untreatable enterococcal infections. The studies will provide new and clinically useful information because it has not previously been appreciated that enterococci possess a capsule which could be used as a vaccine antigen. The development of a serotyping system for enterococci will be the necessary basis for the application of these antigens to immunotherapy and immunoprophylaxis regimens. The molecular studies will focus on the role of capsular polysaccharides of enterococci in pathogenesis, using animal models relevant to important human enterococcal infections, and the genetic mechanisms involved in capsule production. The immunologic studies will focus on determining whether the isolated antigens are targets for protective immune responses. At the end of these studies, we expect to have a better understanding of the immunology of capsular polysaccharides of enterococci, a definition of their role in the pathogenesis of specific enterococcal infections, and their potential for the development of immunotherapies to prevent and/or treat infection in hospital patients.



**Grant:** 1R01AI050669-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** PAYNE, SHELLEY M PHD MICROBIOLOGY, OTB  
**Title:** V. cholerae iron transport mechanisms and pathogenesis  
**Institution:** UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX  
**Project Period:** 2002/01/15-2006/12/31

DESCRIPTION (provided by applicant): Most bacterial pathogens have an absolute requirement for iron. The low availability of iron in most environments has led to the evolution of high affinity iron transport systems. Although iron acquisition systems have been identified in several gram negative organisms, the sources of iron used and the relative contribution of the different systems in their growth and survival in the host and in different niches in the external environment are not understood. *Vibrio cholerae*, the causative agent of cholera, is responsible for considerable morbidity and mortality worldwide. This organism is amenable to genetic manipulation, and several iron acquisition systems have already been identified. However, genetic analysis indicates that there are additional high-affinity iron transport systems in *V. cholerae*. The recent completion of its genome sequence will allow us to identify the remaining iron acquisition systems and to rigorously examine the roles of the systems in different environments and during exposure to different environmental stresses. Our first Specific Aim is to complete our characterization of *V. cholerae* heme transport and utilization. Our genetic data indicate that this pathogen expresses multiple heme transport systems, and we will define which genes are required for heme transport. We will also continue characterization of genes that function in the utilization of heme after it has been transported into the cell. Our second Specific Aim is to identify the transport systems used for the uptake of two exogenous siderophores used by *V. cholerae*, enterobactin and schizokinen. The third Specific Aim is to use our mutant collection, together with other reagents, to determine which transport systems are used during specific environmental conditions, and during growth in the vertebrate host.

**Grant:** 1R01AI050678-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** CHEUNG, AMBROSE L MD MEDICINE  
**Title:** Binding and regulation mechanism for S aureus  
**Institution:** DARTMOUTH COLLEGE HANOVER, NH  
**Project Period:** 2001/12/15-2006/11/30

DESCRIPTION (provided by the applicant): S.aureus is a major pathogen in the community and hospital settings. Because of emerging antibiotic resistance, there is a need to develop new antimicrobial strategies. We have targeted global regulatory elements as a way of developing novel antimicrobial agents. We have identified a regulatory locus called sarA that is involved in the control of several extracellular and cell wall virulence determinants. The sarA locus is composed of three overlapping transcripts, all encoding the SarA protein (14.5 kDa), the major sarA regulatory molecule. Additionally, we recently purified a 13 kDa protein, designated SarR, that binds to the sarA promoter region to down-regulate SarA expression. In searching the S. aureus genome, we discovered a family of SarA-like proteins. Recent crystal structure of SarR (PNAS in press) revealed a dimeric structure as has been proposed for SarA. The structure reveals that SarR represents a new functional class of the "winged helix" family, accommodating an usually long stretch of DNA (about 27 nt) with multiple bending points, eventuating to bending or shortening of target DNA. Based upon the homology and the structural data, we hypothesize that the SarA family of proteins may have homologous structures with similar DNA-binding motifs. Besides structural studies, another major goal of our proposal is to understand the mechanism of gene activation or repression in the SarA family of proteins, using SarA and SarR as models. As such, we have the following aims: I) crystallization of SarR with sarA promoter fragments; II) analysis of the mode of binding of SarA and SarR to the sarA promoter including mutation analysis to precisely map the DNA binding site; III) mutation analysis of SarR to determine the specific activation (or functional) domain; IV) structural and functional analysis of SarS, another SarA homolog; V) deciphering the mechanism of activation and repression of SarR and SarA by examining its interaction with RNAP. In completing these studies, we will be able to determine if the SarA family of proteins possesses conserved secondary structures and binding domains. The SarA family is known to have both activation and repressive functions. Recognizing that both SarA and SarR bind to the UP element to which RNAP interacts, these studies will determine if activation or inhibition of RNAP, by virtue of protein-protein interactions between transcription factor and RNAP, will provide an explanation for the different modes of action for the SarA family. An understanding of these mechanisms will facilitate the development of novel agents that interfere with the function of the SarA family and the ensuing synthesis of cell wall adhesins and toxins.

**Grant:** 1R01AI050725-01A1  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** BUTTARO, BETTINA A PHD  
**Title:** Peptide Pheromones in Enterococcal Plasmid Maintenance  
**Institution:** TEMPLE UNIVERSITY PHILADELPHIA, PA  
**Project Period:** 2002/07/01-2007/04/30

DESCRIPTION (provided by applicant): *Enterococcus faecalis* plays a major role in the current antibiotic resistance crisis. It is one of the leading causes of antibiotic-resistant nosocomial infections and, as part of the normal flora of the intestinal tract, also serves as a reservoir of antibiotic resistance genes. Peptide pheromone-induced conjugative plasmid transfer contributes to the spread of these antibiotic resistance genes and of other enterococcal virulence factors. The focus of this research is on the role of peptide signaling in plasmid maintenance, specifically of the tetracycline-resistance plasmid pCF10 in *Enterococcus faecalis*. The original observations of pheromone signaling in conjugative plasmid biology found that the peptide signal cCF10 produced by plasmid-free recipient cells is used for the induction of conjugative pCF10 transfer. cCF10 is produced at the cell surface and internalized by pCF10-containing donor cells by interaction of the cCF10 receptor with the oligopeptide permease. Once inside the cell cCF10 interacts with intracellular effector molecules to induce conjugation. Recently, we have found a second role, for donor-cell produced cCF10, in maintaining pCF10 in the *Enterococcus faecalis* cell population. In a donor cell population, cCF10 is also produced and its internalization is required to maintain pCF10 in the apparent absence of conjugation. The maintenance functions occur with a minimum pCF10 replicon construct that is unable to be conjugatively transferred, suggesting the role of cCF10 in pCF10 maintenance is distinct from its role in conjugative transfer. The main goal of the research proposed in this application is to gain a basic understanding of how cCF10 is affecting pCF10 maintenance. (1) Does cCF10 act as a pheromone in maintenance of pCF10? (2) Are the oligopeptide permease and the cCF10 receptor PrgZ required for efficient transport of cCF10 (produced extracellularly)? (3) What are the basic features of the pCF10 replicon? (4) What are the intracellular effector molecules responsible for cCF10-dependent pCF10 maintenance? This will lay the groundwork for future experiments to determine how the same peptide can be used for plasmid maintenance without inducing detectable levels of conjugation.

**Grant:** 1R01AI050732-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** FLYNN, JOANNE L  
**Title:** CD4 T Cells in Tuberculosis  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA  
PITTSBURGH  
**Project Period:** 2002/04/01-2007/03/31

CD4 T cells have long been recognized as crucial component of the protective immune response to *M. tuberculosis*. HIV+ subjects have a greatly increased susceptibility to tuberculosis, and mice deficient in CD4 T cells are impaired in their control of *M. tuberculosis* infection. It is generally believed that the major effector mechanism of CD4 T cells in tuberculosis is the production of IFN-gamma for activation of macrophages and subsequent killing of intracellular organisms. Although IFN-gamma production is undoubtedly essential to control of this infection, CD4 T cells likely have other roles in the protective response to *M. tuberculosis*. In a murine model of chronic tuberculosis, depletion of CD4 T cells resulted in fatal reactivation of the infection. However, overall levels of IFN- gamma in the lung were unchanged, and inducible nitric oxide synthase (NOS2) expression and activity were similar to control mice. Thus, the chronic *M. tuberculosis* infection was not contained in the absence of CD4 T cells, even in the face of wild type levels of IFN-gamma and reactive nitrogen intermediates. These data suggested the presence of CD4 T cell-mediated effector mechanisms in addition to IFN-gamma and activation of RNI production by macrophages. This proposal focuses on identifying the roles that CD4 T cells play in host defense against *M. tuberculosis*, apart from IFN-gamma production. Aim 1 focuses on a role for CD4 T cells in maintaining a functioning CD8 T cell response in the lungs. Aim 2 is an investigation of the role of CD4 T cells in macrophage activation, focusing on known potential components, including the relative importance of IFNgamma, CD40, and NOS2. Aim 3 focuses on the spatial relationship of T cells and macrophages within the granuloma, and how the loss of CD4 T cells affects this cell-cell interaction. Finally, Aim 4 is a broad approach to identifying previously unknown CD4 T cell- mediated antimycobacterial mechanisms in in vitro and in vivo models, using gene expression technology. This collaborative project takes advantage of the availability of mouse strains and reagents, as well as the Principal Investigators' previous collaborative work and extensive experience in acute and chronic murine models of tuberculosis. The results obtained are relevant to vaccine design and efficacy, as well as to understanding treatment and prevention of tuberculosis in HIV+ (and therefore CD4 T cell deficient) individuals.

**Grant:** 1R01AI050825-01A1

**Program Director:** TAYLOR, CHRISTOPHER E.

**Principal Investigator:** DERETIC, VOJO P PHD MOLECULAR BIOLOG  
OTHER

**Title:** Hyperacidification and Pseudomonas infections in CF

**Institution:** UNIVERSITY OF NEW MEXICO ALBUQUERQUE, NM  
ALBUQUERQUE

**Project Period:** 2002/06/20-2007/05/31

Cystic fibrosis (CF) is the most common inheritable lethal disorder in Caucasians. The main cause of high morbidity and mortality in CF are the recurring *Pseudomonas aeruginosa* infections and associated inflammation. A clear connection between the genetic lesion in CF and *Pseudomonas* infection has not been unequivocally established. CF is caused by mutations in the CFTR gene, which encodes a chloride channel that has pleiotropic effects on transport of other ions in epithelial cells. Using a novel pH-sensitive GFP technology, we have recently reported that trans-Golgi network (TGN) is hyperacidified in CF respiratory epithelial cells. We hypothesize that dysfunction of this main cellular biosynthetic and sorting organelle leads to defects in CF respiratory cells contributing to the initiation of bacterial infection. We hypothesize that at least one manifestation of the previously unanticipated lower than normal pH in the TGN of CF cells is the well known glycosylation defect including undersialylation of cell surface glycoconjugates which act as receptors for increased *Pseudomonas aeruginosa* binding. In addition, we have observed that another intracellular organelle, the cellubrevin-labeled recycling endosome, is also hyperacidified in CF respiratory epithelial cells. We hypothesize that the dysfunctional recycling endosome in CF may affect various events following bacterial adhesion, such as intoxication of host cells and bacterial uptake and elimination by host cells. In addition, a defective endosomal pathway may result in an overabundance, overstimulation, or defective downregulation of proinflammatory receptors on CF epithelial cells. The aims of this proposal are: 1) To delineate the molecular mechanisms leading to the hyperacidification of TGN and cellubrevin endosomal compartments in CF. 2) To investigate how hyperacidification of TGN in CF affects interactions of respiratory epithelial cells with *P. aeruginosa*. 3) To investigate how hyperacidification of endosomal compartments in CF influences interactions of respiratory epithelial cells with *P. aeruginosa* and whether it plays a role in increased inflammation. In addition, as a part of all three aims, we will determine whether normalizing the pH of intracellular compartments in CF corrects interactions with *P. aeruginosa* and suppress inflammation. These studies are expected to establish a connection between the CFTR defect and infection and inflammation in CF, and provide a basis for development of new chemotherapies using appropriately formulated antacids or inhibitors of pumps and ion channels.

**Grant:** 1R01AI050843-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** BEVINS, CHARLES L  
**Title:** In vivo Models of Defensin Activity  
**Institution:** CLEVELAND CLINIC FOUNDATION CLEVELAND, OH  
**Project Period:** 2002/02/01-2007/01/31

DESCRIPTION (provided by applicant): The discovery of antimicrobial peptides in insects, lower vertebrates and mammals has unveiled a previously unrecognized component of animal host defense. Antimicrobial peptides are gene-encoded antibiotics with activity against many classes of microbes. Defensins are the predominant family of such peptides in mammals. Studies by our group and others have discovered that defensin peptides are expressed by mammalian mucosal epithelial cells, providing them with the capacity to participate in local host defense. Although the intestinal epithelium is a surface in continual contact with luminal contents variably laden with microbes, infection is uncommon. Our underlying hypothesis is that in humans, the epithelial defensins HD5 and HD6 contribute to antimicrobial defense of the enteric mucosa. In this grant proposal, we will test biological functions of epithelial antimicrobial peptides in vivo through transgenic expression of HD5 and HD6 peptides in mice. We propose that transgenic expression of these human defensins may provide mice with an enhanced capacity to resist bacterial challenges. Based on preliminary studies of our established transgenic mice, the experiments described here will establish a clearer understanding of the contributions of human antimicrobial peptides to innate host defense. To test our hypotheses, Aim 1 will assay the immunological consequences of human HD5 expression in transgenic mice. We will characterize the ability of HD5 transgenic mice, compared to control wild-type mice, to resist enteric infection by *Salmonella typhimurium* (Aim 1A), and parallel experiments will extend to other enteric pathogens (Aim 1B). We will examine the impact of transgenic HD5 expression on resident microflora of the mouse intestine (Aim 1C). The antimicrobial activity contributed by transgenic expression will be quantitated in vitro, including analysis of isolated crypts (Aim 1D). The HD6 gene and peptide share little sequence identity to HD5, yet they are expressed together in Paneth cells. In Aim 2, we will generate HD6 transgenic mice (Aim 2A) use recombinant HD6 to develop an antibody for immunoassays (Aim 2B), and characterize the transgenic expression of HD6 at the gene and protein level (Aim 2C). We will then characterize the effects of transgenic HD6 expression on resistance to enteric bacterial colonization and infection (Aim 2D) using the approaches developed in Aim 1. Finally, through lineage interbreeding we will create HD5/HD6 compound transgenic mice to determine if these two peptides have synergistic activities in vivo (Aim 2E). The proposed investigations, and other studies of innate immunity, may provide insights yielding novel therapeutic targets and approaches to combat infectious disease.

**Grant:** 1R01AI050855-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** WALKER, SUZANNE L PHD  
**Title:** Synthesis as a Tool to Understand Ramoplanin and Nisin  
**Institution:** PRINCETON UNIVERSITY PRINCETON, NJ  
**Project Period:** 2001/12/01-2006/11/30

DESCRIPTION (provided by applicant): Antibiotic resistance has increased dramatically in recent years and it is important to develop new strategies to treat bacterial infections. We propose a set of experiments to shed light on the molecular mechanisms by which two unusual substrate binding antibiotics, ramoplanin and nisin, kill bacterial cells. These molecules have very different structures, but both apparently target Lipid II, the disaccharide subunit of peptidoglycan. A better understanding of how these antibiotics function could lead to the ability to design improved antibiotics. The aims of the grant are summarized below. A) To define the structural requirements for substrate recognition by ramoplanin and nisin using synthetic Lipid I/II substrate analogues. This work will provide crucial information about the selectivity of these molecules for carbohydrate moieties found on bacterial cell surfaces. B) To develop selective chemistry to modify ramoplanin; and to evaluate modified ramoplanin analogues for biological activity, Lipid II binding, and ability to form fibrils upon binding to Lipid II. This work will shed light on the mechanism of ramoplanin; furthermore, information about where ramoplanin can be modified without interfering with activity will be useful for the design of other experiments to probe mechanism. C) To determine the structure of ramoplanin in non-aqueous solvents. This work may shed light on the bioactive conformation of ramoplanin and/or on how ramoplanin molecules self-associate when they bind Lipid II. D) To develop a synthetic approach to the synthesis of the N-terminal half of nisin and to evaluate nisin fragments for binding to Lipid II analogues. This work will shed light on the relationship between nisin structure and binding activity. It will also lay the groundwork for the design of hybrid antibiotics. E) To explore the feasibility of designing hybrid antibiotics targeted to bacterial cells by attaching the Lipid II binding domain of nisin to the pore forming antibiotic magainin.

**Grant:** 1R01AI050940-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** SOKURENKO, EVGENI V MD  
**Title:** Dynamic Properties of Bacterial Adhesions  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/09/15-2006/06/30

DESCRIPTION (provided by the applicant): The main goal of the proposal is to develop a comprehensive structural picture of how mechanical force affects the functional state of microbial adhesions. Specific adhesive proteins enable bacteria to recognize ligands leading to the adhesion and colonization of various living hosts or environmental niches, and finally infection. A growing number of experimental observations indicate that mechanical forces generated by shear-flow of body fluids are modulating the affinity and selectivity of adhesins to their ligands. In order to test the extent to which mechanical forces may alter the structure and thus the functional states of adhesins, we propose to characterize the dynamic properties of the most common type of bacterial adhesin - FimH -that is a lectin-like adhesive subunit of type 1 (mannose-sensitive) fimbria of Enterobacteria and Vibrio. In the course of our preliminary studies we have identified distinct structural variants of the Escherichia coli FimH adhesin where shear-flow can induce their preferential binding to target cells, obviously by switching their specificity between the mono-mannoside and tri-mannoside receptors. To develop structural hypotheses how mechanical forces acting on the binding site may affect the tertiary structure of FimH, we have been and will be conducting steered molecular dynamics simulations in which tension is applied between the receptor-binding residues and the C-terminal end of the FimH lectin domain. Deriving a comprehensive understanding of the structure-function relationship of adhesins under static and dynamic conditions requires that molecular biology tools are employed in concert with X-ray crystallography and novel powerful nano-analytical tools to probe, characterize and simulate non-equilibrium protein structures as they relate to function.



**Grant:** 1R01AI050952-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** FOX, JAMES G DVM VET  
MEDICINE:VETERINARY  
MEDICINE-UNSPEC  
**Title:** Microecology-murine gut-initiation & progression of IBD  
**Institution:** MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA  
TECHNOLOGY  
**Project Period:** 2002/04/01-2006/03/31

DESCRIPTION (provided by applicant): Genetically engineered mice with immune dysregulation have been used with increasing success in attempting to unravel and comprehend IBD in humans. IBD is considered to be the result of a combination of genetic and environmental factors. Microbial flora are undoubtedly an important component of the disease process, in which microbial antigens are thought to initiate and promote inflammation, particularly in the presence of immune dysregulation or an impaired mucosal barrier in the susceptible host. The understanding of the complex microecology of the distal intestine is extremely limited because of the tedious nature of identifying individual bacterial species and strains by conventional methods, and the inability to culture many fastidious commensal organisms. To circumvent this lack of bacterial speciation, gnotobiotic animals colonized with known microbiota have been used to great advantage. A standardized microbiota used in colonizing germfree rodents referred to "Altered Schaedler Flora" (ASF) was developed. Because of the limitations of an in vivo monitoring system used to identify the 8 anaerobic bacterial species in ASF, we recently characterized their phylogenetic positions relative to known bacteria by utilizing 16S rRNA sequence analysis. This proposal will use molecular techniques, based on quantitative PCR of ASF 16S rRNA, to screen the microbial diversity of the murine gut and ascertain how microflora dynamics, under defined experimental conditions, with and without helicobacters, influence initiation and progression of IBD in the mouse. Specifically we will 1) characterize, using molecular techniques, the identification, quantification and distribution of Altered Schaedler's Flora (ASF) in the gastrointestinal tract of the IL-10<sup>-/-</sup> and the C57BL mouse. 2) Determine how enteropathogenic *Helicobacter* spp. associated typhlocolitis in the IL-10<sup>-/-</sup> mice alters the microecology of the lower bowel and correspondingly, ascertain whether and how individual species in ASF influence the progression or attenuation of chronic intestinal inflammation. 3) Determine microbial dynamics of ASF after physiological and anatomical perturbation of the lower bowel in mice as well as altering host genotype and how these changes influence subsequent induction of typhlocolitis and possibly colonic adenocarcinoma induced by *Helicobacter* spp. 4) Determine perturbations of ASF and *Helicobacter* spp. prior and subsequent to oral vaccination with *Helicobacter* spp. antigens and mucosal adjuvants, elucidate how these vaccine strategies influence microflora dynamics and impact gut cytokine responses.

**Grant:** 1R01AI051242-01A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** ERNST, JOEL D  
**Title:** Initiation of the Immune Response to M. tuberculosis  
**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO  
SAN FRANCISCO, CA  
**Project Period:** 2002/09/01-2002/12/31

DESCRIPTION (provided by applicant): Tuberculosis(TB) remains a common cause of death, and is the most common copathogen in HIV-associated deaths worldwide. The limitations of drug therapy have increased the interest in developing new vaccines for TB, and improved understanding of immunity to TB is essential for development of improved vaccines. While a role for T lymphocytes in immunity to TB is well established, the mechanisms of initiation of adaptive immunity to M. tuberculosis(Mtb) are not well understood. In particular, the specific contributions of macrophages and dendritic cells to initiation of immunity to Mtb are not known. We have found that immunity to Mtb in mice depends on recruitment of macrophages and dendritic cells to the lungs and to the mediastinal lymph node, which drains the lungs. We have also found that mice with a dendritic cell-selective defect in MHC class II antigen presentation exhibit defective CD4+ T cell responses to Mtb. We hypothesize that macrophages and dendritic cells play distinct roles in initiation of immunity to Mtb, that dendritic cells transport intact Mtb from the lung to the mediastinal lymph node, and that the mediastinal lymph node plays a dominant role in the immune response to Mtb. We will use novel procedures and mutant mice to test these hypotheses. We will characterize the immune response to Mtb in mice with a dendritic cell-selective defect in class II antigen presentation, to determine whether dendritic cells are essential for initiation of immunity to Mtb. We will use immunohisto chemistry and flow cytometry, with Mtb expressing heterologous markers, to determine the rates of infection of macrophages and dendritic cells in the lung, mediastinal lymph node, and spleen, and will test the hypothesis that dendritic cells transport Mtb from the lung to the mediastinal lymph node, by a mechanism that depends on the chemokines CCL19 and CCL21, and their receptor, CCR7. We will also quantitate the CD4+T cell response in the mediastinal lymph node and spleen, to determine the relative contributions of these organs to TB immunity. We will perform in vitro studies to determine whether dendritic cells are uniquely able to prime naive T lymphocytes to respond to antigens expressed by Mtb, and we will test the hypothesis that macrophages modify dendritic cell-dependent initiation of immunity to Mtb. These studies will provide a foundation for studies to target candidate vaccines to specific antigen-presenting cells, and will provide a basis for studies of variable immune responses to natural infection with M. tuberculosis.

**Grant:** 1R01AI051251-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** NASER, SALEH A PHD  
**Title:** Mycobacterium avium subspecies in Crohn's Disease  
**Institution:** UNIVERSITY OF CENTRAL FLORIDA ORLANDO, FL  
**Project Period:** 2002/06/01-2006/05/31

Crohn's disease (CD) is a debilitating chronic inflammatory bowel disease characterized by patches of inflamed tissue. The underlying cause of inflammation and provocation of the immune response in CD patients has yet to be determined. In theory, the immune system usually reacts against an invading organism such as an insect bite or bacterial infection, or is over-sensitive, as in allergic reactions to grass pollen etc. These reactions cause irritation and pain in the affected area, which subside when the immune system has dealt with the potential threat. Defects in the immune system of CD patients have been reported, both in the ability of the cell to phagocytose and in immune killing after phagocytosis. The cytokine pattern in CD is Th1-like and defect in the ratio of proinflammatory to anti-inflammatory cytokines has been proposed. A specific antigenic stimuli has not been identified, but pathogenic bacteria such as Mycobacterium avium subsp paratuberculosis (MAP) and specific invasive E. coli strains have been proposed. In addition, autoantibodies derived from molecular mimicry from bacterial antigens, or from host origin may also be causative agents of the inflammatory lesions in CD. Defects in the ability of macrophages to present antigen to T-cells and B-cells may also have a role. The mycobacterial theory is based on the significant similarity between CD and Johne's disease, a chronic enteritis in cattle that is caused by MAP. The two diseases share histological and pathological characteristics similar to those in tuberculosis and sarcoidosis. It is believed that MAP may be causing an immune reaction in the gut, resulting in a continuous immune response, which gets better and worse as the number of bacteria increase and decrease. Another possibility is that some parts of MAP like the heat shock proteins similar to parts of the gut lining resulting in triggering an immune response: a process known as autoimmunity. Finally, there may be defects in the immune reaction to MAP or proteins in the gut. In this case, the immune cells fail to deal with the invading organism, which is able to persist in the tissues, causing further inflammation. Many studies have been performed in an attempt to investigate a mycobacterial role in the etiology of CD and its pathogenesis. The outcome has been inconsistent which has added to the controversy. The role of MAP, if any, in the etiology of CD has become increasingly debated in recent years causing a need for clear elucidation. While positive results would change the course of therapy and investigation in CD, a negative result will go a long way toward clearing up the MAP debate. In this study, our team will investigate the overall role of MAP, if any, in CD etiology by addressing the following questions: Is MAP present in CD lesions? Is it culturable? Can MAP be identified using PCR, RT-PCR or fluorescence in situ hybridization (FISH) techniques? Is there any immune reaction activity against MAP in CD patients? Is it cellular, humoral or both? What types of immune cells are present in CD lesions compared to non-inflammatory tissue or tissue from non-IBD and healthy controls? Are there any abnormalities in bacterial phagocytosis by peripheral blood monocytes and neutrophils from CD patients compared to normal cells? Are there factors inhibitory to phagocytosis in CD serum? Are there any abnormalities in antigen presentation and lymphocyte transformation to recall antigens from MAP? Are there any

inhibitory or augmenting factors present in the serum from CD patients (cellular and serum crossover)? Our approach in this project is to determine if MAP or reactions against MAP are present in full thickness surgical tissue, heparinized blood and sera specimens from patients with CD using well-developed methodology in the fields of microbiology, immunology and molecular microbiology. We will investigate the presence of MAP in tissue specimens directly by using nested PCR, RT-PCR and FISH and indirectly by culture using a newly developed culture media appropriate for isolation of cell wall deficient form of MAP. We will also investigate the humoral immune reaction in CD sera using p20 antigen, a MAP specific protein. Additionally, the type and state of immune cells will be determined in inflamed versus non-inflamed tissue specimens from CD patients. We will also examine how these cells from CD patient blood are able to ingest and kill MAP, and whether this ingestion results in a normal immune response. This is the first study designed to comprehensively examine the overall presence/absence of MAP in CD tissue and the immune response in CD patients. The results will give us a better idea as to whether MAP causes CD, or whether there is an inherent defect in the immune system, which allows bacterial persistence or autoimmunity to occur in the gut. Ultimately, the outcome of this study will go a long way toward clearing up the MAP debate.

**Grant:** 1R01AI051259-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** RELMAN, DAVID A  
**Title:** Crohn's Disease: Microflora Analysis and Host Response  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 2002/09/01-2006/06/30

DESCRIPTION (provided by applicant): The long-term objectives of this application are to identify microorganisms as well as human cellular pathways that promote Crohn's disease, and to develop novel diagnostic tools and therapeutic strategies for this disease. The short-term objectives include the identification of microflora and host expression patterns that are associated with disease activity, and assessment of molecular methods for pathogen identification. The specific aims are: Aim 1: Identify bacterial and archaeal species associated with active Crohn's disease using broad range ribosomal DNA PCR, laser capture microdissection, and fluorescent in situ hybridization. Tissues with ulcerative colitis, inactive Crohn's disease, and no apparent disease will serve as some of the controls. Aim 2: Quantify differences in relative abundance of bacterial and archaeal species found in Crohn's disease and controls. An rDNA microarray will be used, as well as a more traditional slot-blot hybridization approach. Aim 3: Identify global host gene expression patterns in intestinal tissue and peripheral blood that are associated with Crohn's disease. Expression patterns will be defined that correlate with disease state and activity, and with bacterial and archaeal microflora profiles. State of the art, high-density human cDNA microarrays, and both unsupervised and supervised pattern recognition algorithms will be used to reveal disease-associated signatures. This combination of approaches offers opportunities for characterizing Crohn's disease, and for examining the complex interactions of human host and microbial flora during states of health and disease.

**Grant:** 1R01AI051282-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** NEISH, ANDREW S MD  
**Title:** Epithelial Responses to Enteric Organisms  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 2002/05/15-2006/04/30

DESCRIPTION (provided by applicant): Bacteria are capable of establishing a wide variety of interactive relationships with eukaryotic hosts that may be symbiotic, commensal or parasitic. In humans, such parasitic relationships result in both overt and covert disease. One site where prokaryotic-eukaryotic interactions are particularly diverse and clinically relevant is in the mammalian intestinal tract, where a vastly complex ecosystem of bacteria interfaces with an immense epithelial surface. It has become apparent that both host and microbe influence each other's physiological function to arrange a generally, though not absolutely, mutually beneficial coexistence. Clinical syndromes such as idiopathic inflammatory bowel disease may result when this mutual tolerance breaks down. Furthermore, some bacteria have evolved lifestyles that directly or indirectly elicit host responses characteristic of tissue injury, thus these organisms are generally considered pathogens. A classic example is the common Gram negative enteropathogen *Salmonella*. These organisms are causal of a variety of clinical syndromes, including inflammatory diarrhea, systemic typhoid fever, reactive (non-infectious) arthritis and potentially, other previously unrecognized, medically important manifestations. Recent technical developments have permitted large-scale, parallel analysis of gene expression, or "expression profiling". These methods allow genome-wide analysis of regulatory programs elicited by given stimuli. In this proposal we will employ the approach of infection/colonization with bacteria. For most of our proposed studies, we will utilize *Salmonella typhimurium*, for which we have characterized a spectrum of genetic and environmental variables that affect virulence. We will analyze other strains of *Salmonella*, both pathogenic and non-pathogenic, with the overall goal of defining a host "expression profile" of bacterial pathogenesis that will be of great utility in the study of host interactions with other pathogens. More significantly, these data will be invaluable in the recognition of these signatures in human diseases potentially associated with infection by known and unknown organisms.

**Grant:** 1R01AI051283-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** INAMINE, JULIA M PHD  
MICROBIOLOGY:MICROBIOLOGY-UNSPEC  
**Title:** Molecular Definition of Mycobacterium paratuberculosis  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 2002/06/01-2006/05/31

DESCRIPTION (provided by applicant): This proposal is in response to RFA AI- 01-004 "Infectious Etiology of Chronic Diseases: Novel Approaches to Pathogen Detection" and specifically addresses the study of Mycobacterium avium subspecies paratuberculosis (M. paratuberculosis). M. paratuberculosis is the etiological agent of Johne's disease, chronic granulomatous enteritis in cattle and other ruminants, and has been implicated as a possible cause of Crohn's disease in humans. The difficulty in confirming or refuting an etiological link between M. paratuberculosis and Crohn's disease is a reflection of two issues associated with M. paratuberculosis: 1) the very slow growth of the bacterium engenders a long incubation period in the host and hinders detection; and 2) there is a poor understanding of the biochemistry and genetics of this organism. It is our contention that the second issue can be best addressed by a molecular definition of M. paratuberculosis. This goal will be accomplished by four specific aims: 1) use standard proteomic methods and develop a new ICAT- based method to identify M. paratuberculosis-specific gene expression; 2) perform genomic analyses by using Suppression Subtractive Hybridization to identify M. paratuberculosis-specific sequences; 3) characterize the polysaccharides, lipoglycans and lipids expressed by M. paratuberculosis to provide a complete biochemical analysis that will assist in defining chemical markers for this organism; and 4) develop a proteome website and a reagent repository (including recombinant M. paratuberculosis-specific proteins and clinical isolates) as a service to other basic researchers and clinicians. We will employ the strategies that are currently used by the Mycobacteria Research Laboratories at Colorado State University to support tuberculosis and leprosy research throughout the world to provide a rapid and economic means to obtain information required to develop new diagnostics and vaccines, and elucidate the biochemical and genomic differences that allow M. paratuberculosis to maintain a specific biological niche that is not shared by the closely related M. avium subspecies avium.

**Grant:** 1R01AI051291-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** GOLDBERG, JOANNA B  
**Title:** Helicobacter pylori LPS Signaling and Role in Chronicity  
**Institution:** UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA  
CHARLOTTESVILLE  
**Project Period:** 2002/06/15-2006/05/31

Helicobacter pylori is a bacterial pathogen that chronically colonizes the human stomach. One of the distinctive features of H. pylori is the lipid A moiety of its lipopolysaccharide (LPS). Compared to the prototypical Enterobacteriaceae lipid A, it has a unique structure and is much reduced in its endotoxic activity. Our hypothesis is that H. pylori LPS has different effects on host cell signaling pathways than Escherichia coli LPS. Further we speculate that these differences result in the inability of the human host to respond and clear the H. pylori infection. We will take a genome-wide approach to detect and analyze changes in the human epithelial cell response to H. pylori LPS. In Specific Aim 1, we will use microarray analysis to detect changes in gene expression in gastric epithelial cells treated with H. pylori LPS compared to untreated cells and those treated with E. coli LPS. Whether these changes are due to the recognition by Toll-like receptor 2 (TLR2) or TLR4 will also be assessed using cells either with transfected TLR2 or TLR4, or with dominant negative versions of these receptors. These findings will be confirmed by RT-PCR. In Specific Aim 2, a proteomic approach will be taken; protein profiles of epithelial cells after H. pylori LPS or E. coli LPS treatment will be compared by 2D gel electrophoresis. Proteins of interest will be identified by mass spectrometric analysis. Together these approaches should allow us to determine the specific program of host responses to H. pylori LPS. This information should give us a better understanding of how this organism is able to chronically persist in the stomach and evade eradication by the host immune system. In Specific Aim 3, we will isolate H. pylori strains with alterations in the lipid A structure to determine which portion is responsible for the reduced endotoxic activity. The long-term goal of this research will be to develop a rational approach for new therapeutic treatments for H. pylori infections. Further we anticipate that deciphering the transcriptional and protein profiles resulting from H. pylori LPS will provide us with a framework to recognize the diagnostic features of chronic infections.



**Grant:** 1R01AI051298-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** PACE, NORMAN R PHD BIOLOGY  
NEC:BIOPHYSICS  
**Title:** Molecular Analysis of Microbes in Chronic Bowel Disease  
**Institution:** UNIVERSITY OF COLORADO AT BOULDER BOULDER, CO  
**Project Period:** 2002/09/01-2005/06/30

DESCRIPTION (provided by applicant): This proposed research program will determine the microbial constituents of tissues associated with inflammatory bowel diseases (IBD), in both human and animal systems, in order to identify microbes associated with the disease-states. The human inflammatory bowel diseases, ulcerative colitis (UC) and Crohn's disease (CD), are chronic, devastating diseases of unknown etiology. It is possible that microorganisms, perhaps indigenous and so far undiscovered, participate in the disease process. Although some evidence suggests that *Mycobacterium avium* ssp. paratuberculosis (MAP) may be involved in CD, microbiological analyses have been critically compromised by the clinical need to culture unknown organisms in order to detect and identify them. Culture techniques are frequently ineffective and usually underestimate the true diversity of microbes in natural samples. The phylogenetic analysis of ribosomal RNA (rRNA) genes, amplified from mixed community genomic DNA (e.g. host plus associated microbiota) by polymerase chain reaction, allows species identification in the absence of cultivation. We propose to analyze the microbial communities of both human IBD and normal gastrointestinal samples by this rRNA gene-based technology in order to identify and characterize candidate microbial etiological agents of IBD. The results of molecular studies will guide directed attempts to culture suspected pathogens, including MAP, from diseased tissues. Parallel analyses of animal IBD models, including bovine Johne's disease and rodent models of IBD, will be conducted in order to validate the molecular-phylogenetic strategy, provide insight into microbial involvement in IBD pathogenesis, and guide the choice of appropriate tissues to be analyzed in human IBD.

**Grant:** 1R01AI051334-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** CAMERON, CAROLINE E PHD  
**Title:** Extracellular matrix adhesins of *Treponema pallidum*  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/04/15-2006/03/31

DESCRIPTION (Provided by the applicant): Syphilis, caused by the spirochete bacterium *Treponema pallidum* subsp. *pallidum*, is a chronic bacterial infection that remains a public health concern worldwide, with an estimated 12 million new cases reported in developing nations, Eastern Europe, and the Southern United States. In the absence of appropriate antibiotic treatment, *T. pallidum* establishes a lifelong chronic infection that may progress to the debilitating and potentially fatal tertiary disease in approximately one third of infected individuals. Apart from the serious nature of the disease itself, a number of studies suggest syphilis infections may increase the risk of acquisition and transmission of human immunodeficiency virus. The first step in establishing a *T. pallidum* infection is bacterial attachment and colonization of epithelial surfaces. Consequently, a logical approach for preventing *T. pallidum* infection is to develop methodologies for inhibiting bacterial attachment to host cells. The studies outlined in this proposal focus upon the identification of *T. pallidum* adhesins involved in host cell attachment, and specifically those involved in attaching to components of the extracellular matrix (ECM). The adhesins of *T. pallidum* will be identified using a variety of experimental techniques, including affinity chromatography and expression library screening. Putative adhesins will be expressed in a recombinant form using heterologous expression systems. These proteins will subsequently be investigated for their involvement in host cell attachment by determining their binding potential to host cells and ECM components. Confirmed adhesins will be tested for their ability to complement the non-adherent treponeme *T. phagedenis* biotype Reiter, and the molecular regions of the treponemal adhesins and ECM components responsible for attachment will be identified. The *T. pallidum* adhesins will also be analyzed for their immunoprotective potential in rabbit immunization and challenge experiments, and specifically for their ability to prevent treponemal infection. The long-term objective of the studies outlined in this proposal is to identify *T. pallidum* ECM-adhesins, which will in turn help to further our understanding of the molecules involved in *T. pallidum* pathogenesis and identify potential syphilis vaccine candidates.

**Grant:** 1R01AI051351-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** TUROS, EDWARD PHD  
**Title:** N-Thiolated Beta-Lactams  
**Institution:** UNIVERSITY OF SOUTH FLORIDA TAMPA, FL  
**Project Period:** 2002/03/01-2007/02/28

This project focuses on a very challenging problem in the medical community, the development of treatments for drug-resistant bacterial infections. The leading cause of clinical complications in the United States is nosocomial infections caused by the drug-resistant pathogen, *Staphylococcus aureus*. There is currently a dire need for new drugs to be developed for controlling these infections. Towards this goal, our proposal discusses efforts to identify the biochemical and chemical basis for antibacterial activity of N-thiolated beta-lactams, a new class of antibiotics discovered in our laboratory at University of South Florida. Preliminary data on more than 50 active analogues indicates that these substances possess antimicrobial behavior selective for *Staphylococcus* bacteria, with enhanced activity towards multi-drug resistant strains (MRSA). Unlike all previously known beta-lactam drugs, these compounds appear to affect early developmental events during cell replication, not cell wall crosslinking. These compounds have highly unusual structure-activity profiles which need to be explored further. Electron microscopy experiments indicate that our lactams produce no morphological defects in MRSA cells, or cytotoxic effects in human fibroblasts. The compounds are stable over a wide pH range (pH 1 to 10), and are totally transparent to penicillinases as well as to most chemical reagents we have studied. Our Proposed Studies consist of three Specific Aims. Aim 1 is to study the biochemical and chemical basis for antimicrobial activity, and will include (1) electron microscopy experiments to look for morphological defects in bacterial cells due to damage by the lactams, and to identify where the drug accumulates in the cell, (2) radiolabeling to determine which of the primary cellular processes (cell wall synthesis, protein synthesis, or nucleic acid synthesis) are affected by the beta-lactams, and the means by which the drugs function chemically, and (3) studies to define whether the lactams bind covalently or non-covalently to the biological target, and to identify the cellular target. Aim 2 is to assess further whether the lactams are cytotoxic to mammalian cells. Aim 3 is to develop new approaches to solid phase synthesis of affinity resins (for experiments on isolating the cellular target) and lactam libraries (for expanded drug screening). We also aim to develop novel prodrug delivery systems for the prevention and treatment of MRSA infections. We believe that these studies will provide extraordinary opportunities to develop new therapeutics and approaches for the control of hospital-borne drug-resistant infections.

**Grant:** 1R01AI051405-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** KURT-JONES, EVELYN A  
**Title:** Heat Shock Proteins and Helicobacter Pylori Pathogenesis  
**Institution:** UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA  
WORCESTER  
**Project Period:** 2002/05/15-2007/04/30

DESCRIPTION (provided by applicant): Helicobacter pylori infection causes gastritis, peptic ulcer disease, gastric atrophy and gastric cancer. The World Health Organization has classified H. pylori as a Class I carcinogen. In animal models, the progression of H. pylori disease from superficial gastritis to gastric cancer is related to the severity of the host inflammatory response. The identification of H. pylori components and host factors that contribute to the inflammatory response may lead to important insights into the mechanism of peptic ulcer disease and/or gastric malignancy. Heat shock proteins are potent activators of inflammatory cytokine production. Heat shock proteins produced by bacteria and endogenous heat shock proteins produced by damaged tissue cell accumulate in foci of infection and inflammation. Our recent data indicate that Toll-like receptors and CD14 are important in the innate immune response to bacterial heat shock proteins. In this proposal we will investigate the role of Toll-like receptors in the recognition of bacterial heat shock proteins and in the control of bacterial infection and inflammation. Specifically, we will use in vitro and in vivo approaches to investigate the role of heat shock proteins in H. pylori infection and pathogenesis and development of tumors.

**Grant:** 1R01AI051586-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** MARCONI, RICHARD T PHD  
**Title:** Bdr proteins and Borrelia pathogenesis  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 2002/03/15-2007/02/28

While evidence suggests that plasmid encoded proteins are key in understanding the host-pathogen interaction in Borrelia infections, the functions for most of these proteins remain undefined. Since the plasmid carried genes are largely unique to the Borrelia, it is likely that they hold the key to understanding the unique attributes of Borrelia biology and pathogenesis. The bdr gene family is a large, polymorphic, plasmid carried gene family carried and expressed by all species of the genus. In Borrelia burgdorferi B31MI the gene family contains 18 members distributed among the linear and circular plasmids. We have demonstrated that the Bdr proteins are organized into distinct sub-families, are associated with the inner membrane, and are differentially expressed in response to environmental conditions. In this application we present a strategy for defining the expression patterns of the Bdr proteins in different environments, elucidating the mechanisms involved in their regulation, and for testing the effects of Bdr gene inactivation on Borrelia pathogenesis.

**Grant:** 1R01AI051607-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** OHLENDORF, DOUGLAS H  
**Title:** Structural Genomics of *S. aureus* Pathogenicity Islands  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2002/03/15-2006/02/28

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is a primary human pathogen and a leading cause of hospital-acquired infections (over 700,000 annually), food poisoning, sepsis, and toxic shock syndrome. Currently more than 90 percent of community-isolated strains of *S. aureus* are resistant to penicillin or its derivatives. The ubiquity of *S. aureus* and its ability to rapidly develop antibiotic resistance have prompted monitoring by the WHO, the CDC and others. Understanding the basis for the pathogenicity of *S. aureus* opens the door to the development of new therapeutics to combat infectious diseases produced by this organism. *S. aureus* produces a number of virulence factors. Sequencing of strains of *S. aureus* has shed light into how the genes for these factors are organized. Recent studies have revealed that the genes for a number of the pyrogenic toxin superantigens are located on mobile genetic elements called pathogenicity islands that are about 16 kb in size and flanked by direct repeats. Recent microarray analysis of *S. aureus* pathogenicity island 3 (SaPI3) from strain MN NJ has shown that mRNA is produced for 21 of the 23 ORFs examined. In SaPI3 only 6 of these open reading frames (ORFs) encode for proteins whose sequences are homologous to proteins with a known structural fold. The goal of this project is to use the structural genomics paradigm to investigate the SaPI3 ORFs. If soluble protein cannot be isolated or crystallized for a particular ORF, orthologs from other pathogenicity islands (6 *S. aureus* pathogenicity islands have been identified to date) will be expressed and studied. Functional hypotheses derived from the structures and analyses of ORF null mutants will be tested using assays by the principal investigator and his collaborators. The principal investigator has been working on gram-positive pathogens since 1993. Since then workers in the laboratory have determined the structures of staphylococcal toxic shock syndrome toxin-1 (wild type and 8 mutants), of streptococcal pyrogenic exotoxin A, and of staphylococcal exfoliative toxins A and B. Progress toward the goals to this proposal include cloning 22 ORFs of SaPI3, the production of soluble protein from 7 ORFs and the crystallization of proteins from 3 ORFs.

**Grant:** 1R01AI051621-01  
**Program Director:** GOTTLIEB, MICHAEL  
**Principal Investigator:** STANLEY, SAMUEL L  
**Title:** Structure-Function of Entamoeba Alcohol Dehydrogenase 2  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 2002/05/01-2006/04/30

Nontypeable *Haemophilus influenzae* (NTHi) causes infections in chronic obstructive pulmonary disease (COPD) and otitis media (OM). Both are characterized by inflammation. The molecular mechanisms underlying NTHi-induced inflammation remain poorly defined. Our long-term objective is to understand the molecular mechanisms by which the inflammatory response is induced and regulated in NTHi infections. Our recent studies showed that NTHi strongly activates nuclear factor-kappaB (NF- kappaB) via Toll-like Receptor 2 (TLR2), a newly identified receptor for bacteria. Because TLR2 expression in airway epithelial cells is low and overexpression of TLR2 greatly enhances NTHi-induced NF-kappaB activation, we hypothesize that NTHi up-regulates TLR2 via a specific signaling network. Our preliminary results indeed indicate that NTHi strongly up-regulates TLR2 via a positive NF-kappaB pathway and a negative p38 MAPK pathway. Moreover, glucocorticoids synergistically- enhance NTHi-induced TLR2 up-regulation. These encouraging results have thus laid a solid foundation for further investigation of the molecular mechanisms underlying NTHi-induced TLR2 up-regulation (short-term objective). Aim 1. Determine the contribution of NF-kappaB activation to NTHi-induced TLR2 up-regulation by perturbing NF-kappaB signaling pathways. Aim 2. Determine the contribution of p38 MAPK signaling pathway to NTHi-induced TLR2 up-regulation by perturbing p38 signaling pathway. Aim 3. Determine the signaling mechanisms by which glucocorticoids synergistically enhance NTHi-induced TLR2 up-regulation by studying the effect of glucocorticoids on NTHi-induced activation of p38 pathway. Significance: Understanding the signaling mechanisms underlying NTHi-induced TLR2 up-regulation will not only bring new insights into the regulation of inflammation, but will also open up novel therapeutic targets for modulating inflammatory responses in COPD and OM. Moreover, elucidating the molecular mechanisms by which glucocorticoids enhance NTHi-induced TLR2 up-regulation will provide instructive information regarding how to use glucocorticoids more appropriately in the clinic.

**Grant:** 1R01AI051622-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** BERTOZZI, CAROLYN R AB  
**Title:** Mycobacterial sulfation pathways  
**Institution:** UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA  
**Project Period:** 2002/04/01-2006/03/30

DESCRIPTION (provided by applicant): Sulfated are central mediators of extracellular traffic and cell-cell communication in humans. The enzymes that install and remove sulfate esters, sulfotransferases and sulfatases, respectively, are now appreciated as major contributors to human health and disease. By contrast, the roles of sulfated sugars and the associated enzymes in bacteria remain relatively unexplored. Mycobacterial pathogens have been declared a global emergency by the World Health Organization, particularly in regard to the deadly synergy of *Mycobacterium tuberculosis* with AIDS, but also due to the emergence of drug-resistant strains. In *Mycobacteria*, several sulfated molecules have been identified. These include a sulfated glycolipid, SL- 1 that has been implicated as a virulence factor for *M. tuberculosis*. Another sulfated carbohydrate, part of a glycopeptidolipid, has been detected in a drug resistant strain of *M. avium* isolated from an AIDS patient. Recently, the complete genome sequences of *M. tuberculosis*, *M. avium*, and *M. smegmatis* have become available, enabling the search for genes that participate in sulfation pathways. We have identified an extensive family of sulfotransferases and sulfatases from the completed genomes of these three *Mycobacteria*. The enzymes may be critical determinants of *Mycobacterial* virulence and potential targets for anti-*Mycobacterial* therapy. Through a collaborative effort, our laboratories (Prof. Carolyn Bertozzi and Prof. Lee Riley, UC Berkeley) have initiated a program aimed at the genetic and biochemical characterization, and small molecule inhibition of the sulfotransferases from *M. tuberculosis* and *M. smegmatis*. In addition, we have identified several sulfatases that have considerable similarity to mammalian carbohydrate sulfatases, suggesting a role for these enzymes in host/pathogen interactions. Finally, in order to define the sulfur incorporation pathways of *Mycobacteria*, we have begun the characterization of enzymes involved in the early stages of cysteine biosynthesis. The aims of this proposal are threefold: (1) to determine the functions of the carbohydrate sulfotransferases in *M. tuberculosis* and *M. smegmatis* using genetic, biochemical and chemical approaches; (2) to investigate the involvement of bacterial sulfatases in host/pathogen interactions; and (3) to define the sulfur assimilation pathway of *M. tuberculosis*.



**Grant:** 1R01AI051629-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** REYNOLDS, KEVIN A PHD  
**Title:** CYCLOHEXANECARBOXYLIC ACID AND POLYKETIDE BIOSYNTHESIS  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 2002/03/01-2007/02/28

DESCRIPTION (provided by applicant) Bacterial polyketide biosynthetic processes provide a wide range of structurally diverse bioactive natural products used as immunosuppressants, antibiotics, antiparasitics, growth promotants, and anticancer agents. Tremendous advances in genetic and biochemical understanding of the polyketide synthases (PKSs) and related enzymes involved in these biosynthetic processes has ushered in a new era of drug discovery in which different components of the processes can be combined or altered in order to access novel natural products. An understanding of the unique enzymes, which both provide the unusual biosynthetic precursors used in these processes, and incorporate them into a polyketide product is critical to this effort. One such example is found in ansatriemycin biosynthesis where coenzyme A activated cyclohexanecarboxylic acid (CHC-CoA) is generated from a shikimate pathway intermediate, and subsequently used to generate the final antifungal polyketide product. Combining five CHC-CoA biosynthetic genes from the ansatrienin biosynthesis gene cluster of *Streptomyces collinus* with the avermectin biosynthetic genes in an *Streptomyces avermitilis* host, has resulted in an engineered strain that produces the commercial novel antiparasitic agent doramectin (an avermectin analog generated using CHC-CoA as a biosynthetic precursor). In addition to antiparasitic and antifungal agents, CHC-CoA and related compounds are important precursors used in the biosynthesis of a group of polyketide-derived protein phosphatase II inhibitors, promising antitumor agents, as well as novel functionalized antibacterial agents. The long-term objective of this application is to use the CHC-CoA biosynthetic genes and a hybrid combinatorial biosynthetic approach to generate and ultimately test the biological activity of novel members of each of these different drug classes. This objective will be accomplished through four specific aims. 1) Determination of the rate-limiting step in CHC-CoA biosynthesis and biochemical characterization of the enzymes involved in the pathway. 2) Cloning and sequencing of the biosynthetic pathway genes for formation of CHC-CoA-derived phoslactomycins, members of the important class of antitumor protein phosphatase II inhibitors. 3) Generation of new antiparasitic and antibacterial agents by using the phoslactomycin and CHC-CoA biosynthetic genes as tools to generate bacterial hosts containing hybrid polyketide synthases and CHC-CoA pathway intermediates. 4) Generation of novel protein phosphatase II inhibitors, by manipulation and combinatorial biosynthesis of the phoslactomycin biosynthetic gene cluster.

**Grant:** 1R01AI051677-01  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** SHELLITO, JUDD E MD OTHER AREAS  
**Title:** IL-17 and Klebsiella Pneumonia  
**Institution:** LOUISIANA STATE UNIV HSC NEW NEW ORLEANS, LA  
ORLEANS  
**Project Period:** 2002/05/01-2007/04/30

The long-term objective of this project is to understand interactions between cellular and soluble factors in host defense against bacterial pneumonia. Preliminary data from our laboratory indicate that deposition of bacteria (*Klebsiella pneumoniae*) into the lungs of mice stimulates the release of one such soluble factor- the lymphocyte- derived cytokine, Interleukin-17 (IL-17). Additional experiments in IL- 17 receptor "knock-out" mice show increased mortality and impaired neutrophil recruitment after *K. Pneumoniae* challenge. In this project, we will test the experimental hypothesis that IL-17 released from lung T-lymphocytes amplifies the host response to bacterial infection through stimulated release of C-X-C chemokines for neutrophils and enhanced release of other proinflammatory cytokines. In Specific Aim 1, we will localize IL-17 to CD4+ and CD8+ T- lymphocytes in the interstitium or alveolar space at serial intervals after bacterial challenge using flow cytometry and laser capture microdissection. In Specific Aim 2, we will show that IL-17 enhances host release of CXC chemokines and recruitment of neutrophils in response to *K. Pneumoniae*. We will utilize IL-17 receptor knock-out mice and mice administered a neutralizing IL-17 receptor/FC fusion construct by gene transfer. In Specific Aim 3, we will investigate the interactions between IL-17 and other pro-inflammatory and anti-inflammatory cytokines released into lung tissue in response to *K. Pneumoniae*. These experiments will focus on TNF-alpha, IL-12, IFN-gamma, and IL-10. In Specific Aim 4, we will upregulate IL-17 in lung tissue with gene transfer of the murine IL-17 gene using an adenoviral vector and demonstrate increased bacterial clearance and enhanced recruitment of neutrophils. The results of these experiments will provide new information on how IL-17 participates in host defense against bacterial pneumonia and may lead to novel approaches to augment immune function in the immunocompromised host.

**Grant:** 1R01AI051702-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** MCKINNEY, JOHN D BA  
**Title:** Molecular-genetic analysis of TB persistence and latency  
**Institution:** ROCKEFELLER UNIVERSITY NEW YORK, NY  
**Project Period:** 2002/09/01-2007/06/30

DESCRIPTION (provided by applicant): Tuberculosis (TB) is rivaled only by AIDS as a communicable cause of death. Infection is lifelong and may reactivate following an asymptomatic (latent) interval of variable duration. It is estimated that nearly two billion individuals worldwide have been exposed to *Mycobacterium tuberculosis*. Exposed (tuberculin-positive) individuals have a 5-10% lifetime risk of developing active TB. The risk rises to nearly 10% per year for individuals with HIV/AIDS, indicating a key role for the host immune response in maintaining TB latency. Chemoprophylaxis of latent TB to prevent reactivation requires months of drug therapy, a regimen that many individuals are unwilling or unable to complete. The development of better tools for TB control will hinge on the elucidation of the adaptive mechanisms that allow latent *M. tuberculosis* to persist in the face of host immunity and chemotherapy. Towards that goal, this application is focused on the identification of mycobacterial "persistence factors" and "defense factors" via signature-tagged mutagenesis (STM), a method for simultaneous screening of multiple mutants in mice. Two genetic screens are described. The persistence (per) screen will identify *M. tuberculosis* mutants that are specifically impaired in their ability to persist at later stages of infection in mice. The defense (def) screen will identify mutants whose attenuated phenotype in immune-competent mice is reversed in mice with specific immune deficiencies. These screens will identify mycobacterial genes involved in long-term persistence and defense against host immune mechanisms, respectively. The role of these genes in latent persistence will be assessed in the "Cornell model" of chemotherapy-induced latency and reactivation in mice. Our studies in the mouse will serve as a springboard for linked studies in humans. Relevance to human infection will be explored by analyzing the expression of per/def genes in the lungs of mice and humans via real-time RT-PCR with molecular beacons. Bacterial correlates of protection and pathogenesis will be identified by comparison of gene expression profiles in latent v. active human lesions, respectively. These studies will elucidate the host/pathogen interactions that determine the state of infection-latency or active disease-and may point the way to novel interventions against TB.

**Grant:** 1R01AI052167-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** MILLER, VIRGINIA L  
**Title:** RovA regulon and virulence of *Yersinia enterocolitica*  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 2002/07/01-2007/06/30

DESCRIPTION (provided by applicant): *Yersinia enterocolitica* is a bacterial pathogen responsible for a wide range of clinical syndromes but is primarily associated with gastrointestinal disorders. The enteropathogenic yersiniae have served as important models for the study of bacterial pathogenesis at the molecular level due in large part to their ease of manipulation in the laboratory, the existence of a sequenced genome, and the existence of an excellent murine model of infection. In recent years a number of important paradigms of pathogenesis have emerged from the studies of *Y. enterocolitica* and its companion enteropathogen, *Y. pseudotuberculosis*. Type III secretion systems (TTSS) were first described in these bacteria and the ysc TTSS of yersiniae remains one of the best characterized. In addition *Y. enterocolitica* and *Y. pseudotuberculosis* have served as important models of bacterial invasion, a process primarily encoded by the *inv* gene. In studies to further our knowledge of *inv* and its role in virulence we recently identified a gene, *rovA*, that regulates expression of *inv* both in the lab and during an infection. Virulence studies with a *rovA* mutant indicated that the *rovA* mutant was significantly less virulent than the wildtype strain or the *inv* mutant. Evidence suggests that *RovA* regulates previously undescribed virulence determinants. A more detailed examination of the *rovA* mutant with the host indicated it is defective in its ability to induce production of IL-1 $\alpha$  and its ability to disseminate to deeper tissues. The inability to induce IL-1 $\alpha$  appears to result in the inability of the host to trigger an inflammatory response in the Peyer's patches despite the presence of several other proinflammatory cytokines. Preliminary evidence also suggests that the failure to induce IL-1 $\alpha$  is at least partly responsible for the avirulence of the *rovA* mutant. Based on these results and other information, we hypothesize that production of IL-1 $\alpha$  locally in the Peyer's patches during infection is necessary for the full inflammatory response that in turn affects systemic dissemination and virulence of the bacteria. Therefore by studying aspects of the host-pathogen interaction revealed by analyzing the *rovA* mutant in vivo, and together with studying the *RovA* regulon, we can gain a better understanding of the molecular details of the early host-pathogen interactions that affect virulence. Specifically we propose: (1) Characterization of the IL-1 $\alpha$  response in virulence and dissemination of *Y. enterocolitica*; (2) The role of *RovA* regulated genes in virulence, the IL-1 $\alpha$  response and dissemination; (3) The mechanism of *RovA* regulation of gene expression.

**Grant:** 1R01AI052182-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** STEBBINS, C E PHD  
**Title:** Structural Studies of Bacterial Virulence Factors  
**Institution:** ROCKEFELLER UNIVERSITY NEW YORK, NY  
**Project Period:** 2002/06/01-2007/05/31

DESCRIPTION (provided by applicant): Many animal and plant pathogenic bacteria utilize a similar secretion system, termed type III or "contact dependent," to deliver a battery of bacterial effector proteins into host cells. Salmonella typhimurium uses such a secretion system to inject proteins that manipulate host cellular functions to induce the uptake of the bacterium into the normally non-phagocytic cells of the intestinal epithelium. This process relies on less than ten translocated effectors proteins, which collaborate to induce dramatic membrane ruffling, leading to bacterial internalization by macropinocytosis. The long-term goal of this work is to use structural biology as a foundation for a molecular understanding of the invasion process of this pathogen, and to exploit this information in the identification of potential targets for drug screening. The specific aims of this proposal are (1) to determine structures of S. typhimurium invasion-associated translocated effectors, (2) to determine the co-crystal structures of these factors with their host cell targets, and, finally, (3) to use structure-based mutagenesis to examine the interacting surfaces of these factors in the context of bacterial host cell invasion and cytoskeletal manipulation. This work will thus involve a multidisciplinary approach combining macromolecular X-ray crystallography, biochemical assays, and microbial cell biology. Bacterial infection is and has been a significant cause of death and human suffering. Ominously, our weapons for combating bacterial pathogens are now failing as ever-increasing numbers of microorganisms have developed resistance to greater numbers of our drugs. Furthermore, the increased threat of the use of microbial agents as instruments of war or terrorism has become a very real concern. Therefore, a final aim of these studies will be to use the structural information to aid in selecting targets for the screening of inhibitory compounds that will impair the virulence mechanisms of this pathogen, and to serve as a paradigm for developing similar strategies against other infectious bacterial organisms.

**Grant:** 1R01AI052216-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** KNOX, JAMES R PHD  
**Title:** Oxacillinase and Bacterial beta-Lactam Resistance  
**Institution:** UNIVERSITY OF CONNECTICUT STORRS STORRS, CT  
**Project Period:** 2002/07/01-2004/06/30

DESCRIPTION (provided by applicant): We plan to determine the crystallographic structure of the OXA-1 oxacillinase, a troublesome bacterial beta-lactamase which provides clinical resistance to beta-lactam antibiotics (penicillins and cephalosporins). A member of the less-characterized class D family of serine beta-lactamases, OXA-1 is especially active against the penicillins oxacillin and cloxacillin and is now found in 10 percent of E. coli clinical isolates. The architecture of the beta-lactam hydrolysis site in OXA-1 will be compared with that in a class A penicillinase and a class C cephalosporinase to understand the preferential ability of OXA-1 to hydrolyze oxacillin and cloxacillin. Because the oxacillinases are poorly inhibited by currently used agents such as clavulanic acid, a crystallographic structure will aid in the design of more effective inhibitors. OXA-1 crystals which diffract to very high resolution (1.5 Å) have been examined.

**Grant:** 1R01AI052217-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** CLUBB, ROBERT T PHD  
**Title:** Cell Surface Protein Anchoring in Gram-positive Bacteria  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2002/06/01-2006/05/31

DESCRIPTION (provided by applicant): Research will study the molecular mechanism used to covalently anchor surface proteins to the cell wall of Gram-positive bacteria. Surface proteins play important roles during the pathogenesis of human infections and are covalently anchored by sortase enzymes. The mechanism of sortase-mediated attachment is universally conserved, responsible for attaching up to 30 percent of surface proteins and required for infectivity. Work in this proposal will focus on the SrtA protein from *S. aureus*. We will localize its substrate-binding site, determine the functional significance of amino acids within its active site, and investigate how it is activated by calcium. This work will be complemented by the design, synthesis and in vitro testing of peptide-based inhibitors of SrtA. This will enable the structure of a SrtA-inhibitor complex to be determined to gain insights in the molecular basis of substrate recognition. *S. aureus* encodes a second sortase-like enzyme, SrtB, which has no known function. We hypothesize that SrtB anchors proteins to the cell wall by recognizing a sorting signal that has yet to be identified. We will use a unique biopanning method to determine the full range of amino acid sequences that can be processed by SrtA and to search for a SrtB sorting signal. Finally, we will elucidate the three-dimensional structure of the SrtB protein to reveal conserved structural features within its active site and insights into its function. The results of this work will shed light onto the underlying chemistry of cell wall anchoring, identify new peptide signals that target proteins for cell wall attachment, and may facilitate the development of new therapeutically useful anti-infective agents.

**Grant:** 1R01AI052237-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** WILSON, RICHARD K MA  
**Title:** Comparative Genomics in the Enterobacteriaceae  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 2002/09/01-2005/08/31

DESCRIPTION (provided by applicant): The number of available genome sequences from the bacterial family Enterobacteriaceae is reaching a threshold where comparative genomics can drive hypotheses and experiments. In this project we have selected genomes for sequencing based on their pathogenicity and their taxonomic position. These sequences will help us understand these and other related pathogens by defining their differences and similarities in gene content. (1) The genome sequences of *S. enterica* serovar Paratyphi A (SPA), already sampled to 97 percent coverage, will be completed and annotated. SPA is the second most prevalent cause of typhoid and, like *S. enterica* serovar Typhi (STY), is restricted to humans. Typhi is undergoing genome degradation, perhaps associated with its recent adaptation to a narrow host range; we will determine if Paratyphi A is undergoing similar degradation. *Klebsiella pneumoniae* is a major opportunistic pathogen. We have sequenced this genome to 8-fold coverage; it will be closed, finished and annotated. (2) Cost-effective four-fold sampling (97 percent coverage) will be performed for four genomes: a biotype of *S. enterica* Paratyphi B (SPB), which is the third most prevalent cause of typhoid and is host-adapted to man; *S. enterica* Arizonae (SAR), the most distantly related *S. enterica* that regularly causes disease in humans; *Citrobacter koseri* (CKO) and *Enterobacter cloacae* (ECL) both of which are opportunistic pathogens representing the unsequenced genera within or adjacent to the *E. coli*/*Salmonella*/*Klebsiella* clade. Web-based analysis tools that take into account the incomplete nature of the samples will be used to present these data in comparison to other related genomes. Finally, (3) we have amplified and arrayed the complete open reading frames of nearly every CDS in *S. enterica* subspecies 1, serovar Typhimurium LT2. This resource will be supplemented with new putative CDSs, not found in STM, as these sequences become available from STY, SPA, SPB, and other serovars of *S. enterica*. Thus, we will develop an array that can be used in a wide variety of *Salmonella*, both sequenced and unsequenced, for analysis of expression and of genome content.



**Grant:** 1R01AI052299-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** RUBENS, CRAIG E MD CLINICAL MEDICAL SCIENCES, OTHER  
**Title:** Role of penicillin binding protein 1a in GBS virulence  
**Institution:** CHILDREN'S HOSPITAL AND REG MEDICAL CTR SEATTLE, WA  
**Project Period:** 2002/07/15-2007/04/30

DESCRIPTION (provided by applicant): Group B streptococci (GBS) remain the most significant bacterial pathogen causing neonatal sepsis, pneumonia and meningitis in the USA despite CDC-recommended chemoprophylaxis strategies for preventing infection due to this organism. Apart from the capsule, the factors required for survival of GBS in the host are not well defined. Recently, signature-tagged transposon mutagenesis (STM) was used to identify genes required for growth and survival of GBS in a neonatal rat sepsis infection model. A significant proportion of the avirulent mutants had transposon insertions in genes involved in cell surface metabolism emphasizing the significance of these functions for in vivo survival of GBS. We characterized the most attenuated mutant from the cell-surface metabolism group, which had a transposon insertion in a putative penicillin-binding protein gene (ponA) homologue. Based on sequence homology, the disrupted GBS gene is predicted to code for a class A, high molecular weight penicillin-binding protein (PBP1a), possessing both transglycosylase and transpeptidase activity. These bifunctional enzymes catalyze both the polymerization and cross-linking of bacterial peptidoglycan. The PBP1a gene mutant was significantly attenuated in both competitive index and 50 percent lethal dose assays of GBS virulence in neonatal rats. Additionally, the PBP1a gene mutant displayed a significant defect in resistance to opsonophagocytic killing as measured by in vitro bactericidal assays. Complementation analysis in vivo confirmed that the altered phenotypes observed in the mutant were due to the transposon insertion in ponA. The PBP1a gene mutant had a normal growth rate in vitro, produced wild-type levels of capsular polysaccharide and was otherwise phenotypically identical to the parent strain. We hypothesize that the GBS ponA gene is required for resistance to opsonophagocytic killing in vitro and virulence in vivo. Our investigation seeks to define the role of PBP1a in interactions with the host and virulence of GBS in vivo. Aim 1 will complete analysis of the ponA gene and the prfA gene (penicillin binding protein related factor A), a gene cotranscribed with ponA. A nonpolar prfA deletion mutant will be constructed and subjected to phenotypic analysis. Aim 2 will use genetic and biochemical approaches to define the structure and function of the PBP1a protein. Site-directed mutations that disrupt enzymatic activity of the protein will be introduced into PBP1a. Analysis of these mutants will allow us to evaluate whether enzymatic activity of the protein is required for virulence and resistance to opsonophagocytic killing. Aim 3 will determine the role of the PBP1a protein in resistance of GBS to opsonophagocytic killing by investigating the interaction of aponA mutant with serum opsonins and phagocytic cells. While bacterial cell-wall associated enzymes, including PBPs, have been reported to be required for virulence in numerous animal models of infection, no mechanism has been proposed to explain these observations. These studies will be the first to systematically investigate the role of PBP1a in virulence, will

further our understanding of the pathogenesis of GBS infections, and may identify targets for preventative or therapeutic modalities.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R01AI052308-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** SCIDMORE, MARCI A BA  
**Title:** Chlamydia trachomatis Inclusion Membrane Proteins  
**Institution:** CORNELL UNIVERSITY ITHACA ITHACA, NY  
**Project Period:** 2002/07/01-2007/06/30

DESCRIPTION (provided by applicant): Chlamydiae species are obligate intracellular bacteria that are the most frequent cause of sexually transmitted disease as well as the leading cause of preventable blindness worldwide. Chlamydiae replicate in a non-acidified vacuole, termed an inclusion, which is actively modified by chlamydiae to prevent lysosomal fusion and promote intracellular survival. The molecular determinants that mediate chlamydial pathogenesis are largely undefined primarily due to the inability to manipulate the chlamydial genome. The overall goal of this research is to identify pathogenic mechanisms utilized by chlamydiae to promote and maintain their intracellular survival. Because chlamydiae remain sequestered within a vacuole, all interactions between chlamydiae and their host must be mediated through the inclusion membrane. We have identified Chlamydia trachomatis-specific proteins (IncD/E/F/G) that are localized to the inclusion membrane. Their intracellular localization makes them potential mediators of host-pathogen interactions via direct interactions with host proteins. To achieve our overall goals, we propose to identify biological functions of IncD/E/F/G through identification and characterization of cellular targets of IncD/E/F/G. We have identified mammalian 14-3-3 proteins, as the first and only cellular targets of an inclusion membrane protein, IncG. 14-3-3 proteins are dimeric phosphoserine binding proteins that regulate diverse signal transduction pathways through directed subcellular localization of signaling complexes. Specific Aim 1: Experiments are designed to define biological functions of 14-3-3 IncG interactions and determine whether chlamydiae target 14-3-3 proteins to exploit host signal and vesicular-mediated pathways. First, we will disrupt 14-3-3 IncG interactions through expression of 14-3-3 dominant negative mutants and microinjection of anti-IncG antibodies to examine whether 14-3-3's recruitment to the inclusion functions in exploitation of cellular signal transduction and vesicular-mediated pathways. Second, we will use a combination of fluorescence microscopy, yeast tri-hybrid assays and co-immunoprecipitation experiments to determine whether 14-3-3 proteins recruit additional signaling proteins to the inclusion. And third, we will employ co-immunoprecipitation experiments to determine whether chlamydiae alter 14-3-3-dependent signaling pathways by altering host 14-3-3/ligand interactions. Specific Aim 2 utilizes yeast two-hybrid assays to identify cellular targets of IncD/E/F. Identification of cellular targets of Incs and how these interactions contribute to chlamydial pathogenesis will lead to a better understanding of the complex host-pathogen interactions that facilitate chlamydial intracellular survival.

**Grant:** 1R01AI052337-01  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** MULHOLLAND, EDWARD K MD  
**Title:** Appropriate Pneumococcal Vaccination in Infants in Fiji  
**Institution:** UNIVERSITY OF MELBOURNE MELBOURNE,  
**Project Period:** 2002/09/30-2005/08/31

DESCRIPTION (provided by applicant): Streptococcus pneumoniae (Pnc) is the leading vaccine preventable cause of serious infection in infants. The current Pnc conjugate vaccines are safe, immunogenic, and effective. However, the vaccine is very expensive (approximately USD \$200/infant) so it is unlikely to be affordable for most developing countries. Moreover, as health care access in developing countries may be episodic and unreliable, many children do not receive either complete or timely vaccine courses. Therefore, it is important to investigate affordable and flexible ways to deliver this vaccine, which are safe and effective. A recent World Health Organization (WHO) / Global Alliance for Vaccines and Immunization (GAVI) meeting to address impediments to the introduction of these vaccines in developing countries recognized the need to evaluate other regimens of Pnc conjugate vaccine as an important research priority. This study has been deliberately formulated with that need in mind. The proposed site for this research is Fiji. Although health services are good, Pnc disease, particularly pneumonia, remains the commonest cause of childhood morbidity and mortality. Fiji has good vaccine coverage and was the first Pacific country to introduce Hib vaccine. The arrival of the new, expensive Pnc conjugate vaccine presents a dilemma for Fiji and many similar countries. The expense of this vaccine if used in the recommended 3 or 4-dose schedule would consume a large portion of the health budget. This study has two components, aimed at addressing these two issues: 1. A phase 2 immunogenicity study (involving 750 infants) to evaluate regimens using reduced numbers of doses of Pnc conjugate vaccine, and using timing of dosing and combinations with polysaccharide (PS) vaccine that may be more suited to the epidemiology of Pnc disease in developing countries. 2. An epidemiological study will measure the burden of invasive Pnc disease, pneumonia, and otitis media (OM) in Fiji. This will be part of a global effort to address these issues, and will be used to develop rapid assessment tools for these diseases in developing countries.

**Grant:** 1R01AI052397-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** TZIANABOS, ARTHUR O PHD  
**Title:** Host Response in *S. aureus* Infections: Role of T cells  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2002/06/01-2007/05/31

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is an opportunistic bacterial pathogen responsible for a diverse spectrum of human and animal diseases, including wound infections, osteomyelitis, endocarditis, and bacteremia leading to secondary abscesses in any of the major organ systems. Staphylococcal infections occur most frequently when mucosal barriers are breached, following insertion of a foreign body, or in the presence of other factors that compromise the immune system of the host. Currently, the host response to this organism is poorly understood. The majority of studies to date have focused on the role of PMNs modulating the host response to staphylococcal infections. *S. aureus* is typically classified as an extracellular pathogen that does not directly interact with the host immune system via T cells. However, we have shown that the staphylococcus produces a capsule with both free amino and negatively charged carboxyl groups that mediates its pathogenic potential in an experimental model of intraabdominal abscess formation in a T cell-dependent manner. This capsule can activate CD4+ T cells and induce the production of CXC chemokines, peptides that activate and recruit PMNs to sites of inflammation. The transfer of CD4+ T cells that have been activated by the *S. aureus* capsule promotes abscess formation in naive recipient animals. Based on these data, we hypothesize that CD4+ T cells activated by *S. aureus* capsules are critical in determining the outcome of staphylococcal infections through the release of CXC chemokines that control PMN trafficking to infected sites. This hypothesis will be tested in staphylococcal animal models of subcutaneous abscess formation and surgical wound infection. These models are clinically relevant, low-inoculum murine models of staphylococcal disease that mimic important aspects of human disease. We propose to: 1) Characterize the mechanism by which *S. aureus* synthesizes a capsule with a zwitterionic charge motif; 2) Evaluate the role of the capsular polysaccharide in the interaction between *S. aureus* and the host; 3) Determine the role of T cells in the pathogenesis of and host response to staphylococcal infections; and 4) Characterize the T cell-mediated CXC chemokine response to *S. aureus* infection and its role in regulation of PMN trafficking. Results from the proposed studies should provide insight regarding the under appreciated role of T cells in the pathogenesis and host response in *S. aureus* infections. This information may reveal new strategies for the prevention or treatment of *S. aureus* infections through immunomodulation of the host response to this organism.

**Grant:** 1R01AI052443-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** MOORE, BRADLEY S BS  
**Title:** Type III Polyketide Synthases: Structure and Mechanism  
**Institution:** UNIVERSITY OF ARIZONA TUCSON, AZ  
**Project Period:** 2002/06/15-2007/05/31

A new mechanism of polyketide assembly has emerged in bacteria for the biosynthesis of small aromatic residues that serve as important structural elements in a growing number of biologically active natural products. These small aromatic polyketides are synthesized by homodimeric (type III) polyketide synthases (PKSs) that are phylogenetically and biochemically related to ubiquitous plant PKSs such as chalcone synthase. Thus far, type III PKSs have been shown to be responsible for the biosynthesis of natural products such as 1,3,6,8- tetrahydroxynaphthalene (THN) and the formation of key components of more complex antimicrobial and antitumor natural products such as vancomycin, naphterpin, marinone, and kendomycin. While type III PKSs are architecturally simple, they arguably represent the most sophisticated PKSs mechanistically since embodied within their homodimeric architecture is the catalytic machinery necessary for starter molecule recognition and loading, malonyl- CoA decarboxylation and polyketide chain extension, and ultimately, multiple pathways for termination. Their simple gene and protein architecture makes them amenable for study using a variety of sophisticated approaches including heterologous biosynthesis, in vitro and in vivo biochemical analysis, directed and random approaches towards enzyme engineering, and atomic resolution protein x-ray crystallography. Although the analysis of related plant enzymes is fairly mature, research on the bacterial counterparts is only beginning and can be expected to yield novel, interesting, and potentially important information on these simple condensing enzymes. Moreover, the mechanistic and structural understanding of bacterial type III PKSs is likely to be relevant for the productive reengineering of modular type I and iterative type II bacterial PKSs. With the high resolution three-dimensional crystal structure of the first bacterial PKS, THN synthase from *Streptomyces coelicolor* A3(2), nearly in hand, the stage is set for a comprehensive structural and mechanistic analysis of this new subclass of bacterial PKS. Studies will extend to other bacterial type III PKSs, including those involved in the biosynthesis of the clinically important glycopeptide vancomycin, the broad spectrum antibiotic 2,4- diacetylphloroglucinol, and the antitumor antibiotic marinone.

**Grant:** 1R01AI052455-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** GOLENBOCK, DOUGLAS T  
**Title:** Group B. Streptococci and Toll-like Receptors  
**Institution:** UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA  
WORCESTER  
**Project Period:** 2002/08/15-2007/07/31

DESCRIPTION (provided by applicant): Group B streptococci (GBS) are the leading cause of neonatal sepsis, the third most frequent cause of bacterial meningitis and an increasingly important cause of bacteremia and sepsis in adults in the United States today. Preliminary studies have identified a novel proinflammatory component of GBS, which we have designated GBS-factor (GBS-F). Based on experiments in mice with targeted genetic deletions in Toll-like receptor (TLR) expression and on experiments with engineered cell lines, we have determined that responses to GBS-F require expression of CD14, TLRs 2 and 6, and the Toll-adaptor protein, MyD88. Activation of this receptor complex by GBS-F initiates important signaling events such as the activation of NF-kB, the phosphorylation of MAP kinases, the formation of proinflammatory cytokines, and the intracellular production of the toxic oxidant peroxynitrate. In contrast, although other components of GBS appear to engage TLRs, the exact identity of contributing TLRs is entirely unknown. The overall goal of this proposal is to identify and define components of GBS, focusing first on GBS-F, and their cognate Toll-like receptors (TLRs). We propose to characterize the structure of GBS-F and assess its function in vitro and in vivo. Furthermore, we will determine if TLR2, and related downstream signal transduction molecules, mediate a variety of important innate immune responses to GBS, including leukocyte chemotaxis and the intracellular killing of bacteria. Finally, we intend to determine what other TLRs, and associated signal transduction molecules, are involved in GBS recognition and response. The data learned from these studies should help in the development of rational therapeutic strategies to interfere with the deleterious hyperinflammation triggered by GBS and similar microbial organisms.

**Grant:** 1R01AI052474-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** SCHNEEWIND, OLAF MD  
**Title:** Sortase B-anchored surface proteins of S.aureus  
**Institution:** UNIVERSITY OF CHICAGO CHICAGO, IL  
**Project Period:** 2002/06/01-2007/05/31

DESCRIPTION (provided by applicant): Human infections caused by *Staphylococcus aureus* present a serious therapeutic challenge due to the appearance of antibiotic-resistant strains. The mechanism of surface protein anchoring to the *S. aureus* cell wall is examined as a possible target for anti-infective therapy. Staphylococcal surface proteins are synthesized as precursors with an N-terminal signal peptide and a C-terminal sorting signal, containing a LPXTG motif as well as positively charged residues. The charged residues are thought to retain surface proteins within the secretion pathway, allowing sorting signal cleavage between the threonine (T) and the glycine (G) of the LPXTG motif. The carboxyl-group of threonine is subsequently amide-linked to the amino-group of peptidoglycan crossbridges, anchoring the C-terminal ends of surface proteins to the staphylococcal cell wall. Sortase (SrtA), a membrane anchored transpeptidase of *S. aureus*, is responsible for anchoring surface proteins with a LPXTG motif to the cell wall envelope. We report here the identification of a second sortase (SrtB) in the Gram-positive pathogen *Staphylococcus aureus* that is required for anchoring of a hitherto unknown surface protein with a NPQTN motif. Purified SrtB cleaves NPQTN-bearing peptides in vitro, and a srtB mutant is defective in the persistence of animal infections. SrtB is part of an iron-regulated locus called iron-responsive surface determinants (isd), which also contains a ferrichrome transporter and surface proteins with NPQTN and LPXTG motifs. Thus, cell wall anchored surface proteins and the isd locus appear to be involved in a novel mechanism of iron acquisition that is important for bacterial pathogenesis. This proposal aims to characterize the role of sortase B in anchoring surface proteins to the cell wall envelope and to study the contribution of SrtB-mediated cell wall sorting during infection. Genetic and biochemical experiments aim at the identification of genes or gene products that are required for the anchoring of NPQTN-sorting signal containing surface proteins to the cell wall envelope. Other experiments examine the physiological role of the isd locus in iron transport.



**Grant:** 1R01AI054310-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** BAVOIL, PATRIK M PHD  
**Title:** BIOLOGY OF PHAGE INFECTION IN CHLAMYDIA  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 2002/08/01-2006/05/31

DESCRIPTION (provided by applicant): Chlamydial disease of humans includes predominant ocular, genital and respiratory tract infections, with sequelae ranging from blindness, to female infertility, arthritis and asthma. Chronic infection with the respiratory pathogen, Chlamydia pneumoniae is also associated with coronary heart disease, the number one killer disease of humans. In spite of their public health magnitude, chlamydiae are reputed for their elusiveness as infectious microorganisms to clinicians and molecular biologists alike. This owes to several factors, prominent among which are a unique obligate intracellular developmental lifestyle and the fact that chlamydiae have resisted genetic manipulation to this day. We have isolated a bacteriophage, phiCPG1 from the model Chlamydia psittaci strain ?Guinea Pig Inclusion Conjunctivitis?. A member of the single-stranded DNA microviridae family, phiCPG1 is nearly identical to a ?virtual? phage of C. pneumoniae that was revealed by genome sequence analysis. The infection of an intracellular pathogen by its own parasitic bacteriophage is a unique biological phenomenon, with potentially important implications in infection and disease. Moreover, phages offer unique opportunities for the development of molecular and genetic tools for research. The objectives of this application are therefore to gain a broad understanding of Chlamydia phage biology in the context of chlamydial infection. We will determine the molecular basis of the interaction of the phage with its host and comparatively evaluate gene expression in phage-free and phage-infected bacteria. The availability of well-established models of infection and disease in the guinea pig will allow for the first time to study the impact of phage infection on the natural infection of a vertebrate animal by an obligate intracellular pathogen. Finally, the information gained in these studies will be exploited toward the development of genetic methodologies in Chlamydia.

**Grant:** 1R01AI054338-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** KAPLAN, GILLA PHD  
MICROBIOLOGY:BACTERIOLOGY  
**Title:** Host & Pathogen Determinants of M tuberculosis Latency  
**Institution:** PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ  
**Project Period:** 2002/09/15-2007/06/30

DESCRIPTION (provided by applicant): Our aim is to define the host and bacterial factors that characterize the clinical and microbiologic latency which distinguish human tuberculosis. We hypothesize that in human tuberculosis, persistent/latent infection is associated with a different immune response than active chronic disease. For Mycobacterium tuberculosis to survive in the face of these varying host immune responses, it must alter its physiology through differential changes in gene expression. Thus a particular clinical state associated with a specific state of immunity will elicit a corresponding profile of genes expressed by the infecting organism ("molecular mirror"). We will utilize M. tuberculosis infected lung tissue obtained from surgical resection of TB patients with active disease, or asymptomatic persistent infection, or recurrent disease. These tuberculous lung specimens will be used to characterize the host immune response by histology and immunohistology combined with real time RT-PCR with molecular beacons to quantitate leukocyte gene expression in the lung. Our collaborator, Dr. John McKinney, will use the same tissues to identify the patterns of M. tuberculosis gene expression in each type of disease. In addition, we will use the rabbit model of M. tuberculosis infection to generate animals with either active disease or persistent asymptomatic infection (latency). Rabbits will be infected with clinical strains of M. tuberculosis of differing degrees of virulence to recreate the range of human disease. Tissues from rabbits with each form of the disease will be used to characterize the cellular response to infection in the lung and to elucidate the patterns of M. tuberculosis gene expression associated with either active disease or persistent asymptomatic infection (latency). We will also infect rabbits with M. tuberculosis mutants selected to be defective for persistence in murine models and establish whether these mutants retain their persistence defect in the rabbit model. By combining the results obtained from the rabbit studies and the human studies, we will validate our experimental animal model as a mirror of human disease and use the model to identify the molecular correlates of M. tuberculosis latency.

**Grant:** 1R01AI054361-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** KAPLAN, GILLA PHD  
MICROBIOLOGY:BACTERIOLOGY  
**Title:** Regulation of Early Cytokine Receptors to TB Infection  
**Institution:** PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ  
**Project Period:** 2002/09/15-2007/06/30

DESCRIPTION (provided by applicant): The aim of these studies is to understand how the human protective immune response to tuberculosis (TB) is regulated. Our previous work suggests that the levels of inflammatory cytokines (TNF- $\alpha$  and IL-12) produced early in response to infection with *M. tuberculosis* determine the effectiveness of the Th1 response in the mouse. Clinical isolates of *M. tuberculosis* that fail to induce high IL-12 cause more severe disease (that is, are more virulent). In the mouse IFN- $\alpha$ /Beta (type 1 IFN) appear to downregulate the Th1 cytokine response. We hypothesize that in humans, *M. tuberculosis* clinical isolates that induce low levels of IL-12 and/or high levels of type 1 IFN in monocytes and dendritic cells (DC) will be more virulent, i.e., more likely to cause disease or more likely to cause severe manifestations of disease. In addition, we hypothesize that specific polyketides of *M. tuberculosis* are involved in induction of the differential cytokine response. To test these hypotheses we will ask the following questions: (1) Do specific *M. tuberculosis* clinical isolates differentially induce IL-12 in infected human monocytes/macrophages, thereby affecting the efficiency of the Th1 protective response? (2) Is the maturation and antigen presenting function of human myeloid dendritic cells (DC) differentially regulated by *M. tuberculosis* clinical isolates or by *M. tuberculosis* lipids? (3) Does IFN- $\alpha$ /b down regulate the development of IL-12 dependent Th1 responses in human TB? Do specific *M. tuberculosis* clinical isolates differentially induce IFN- $\alpha$ /b production by human leukocytes? To answer these questions we will examine the human leukocyte-*M. tuberculosis* interaction in vitro using a combination of standard immunological assays (ELISA and lymphocyte proliferation; intracellular cytokine staining, FACS analysis), cell biologic assays (DC maturation) and molecular analyses of the monocyte response to infection (2-D gel electrophoresis with mass spectrometry).

**Grant:** 1R01AI055475-01  
**Program Director:** TSENG, CHRISTOPHER K.  
**Principal Investigator:** NICOLAOU, KYRIACOS C PHD CHEMISTRY:ORGANIC  
**Title:** TOTAL SYNTHESIS OF THIOSTREPTON  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 2002/09/30-2006/03/31

DESCRIPTION (provided by applicant): This grant application describes a program directed towards the total synthesis of the novel thiopeptide antibiotic thiostrepton. Produced by several bacterial strains including streptomyces azureus, this naturally-occurring substance is characterized by a novel molecular architecture which includes three highly complex molecular domains: a didehydropiperidine ring system possessing a quaternary center bearing three carbons and a nitrogen; a potentially sensitive thiazoline ring; and a unique quinaldic acid system. The originally proposed total synthesis will feature not only a highly convergent and flexible approach to the assembly of thiostrepton, but moreover the projected route will end with three novel cyclization reactions to complete the most challenging portions of the molecule. Moreover and given the daring nature of this bold approach an alternative, safer strategy for the construction of the macrocyclic rings is proposed. As such, this work is expected to impact positively the areas of new synthetic methodologies and strategies for complex molecule construction. Furthermore, the use of synthesized fragments and thiostrepton itself as chemical biology tools to probe the interactions of small molecules with RNA should help set the stage for drug discovery programs utilizing small organic compounds to selectively target RNA or RNA-protein complexes. The disease areas likely to benefit most from the proposed investigations are bacterial-caused diseases and malaria.

**Grant:** 1R03AI049780-01A1  
**Program Director:** LAUGHON, BARBARA E.  
**Principal Investigator:** ATKINS, WILLIAM M PHD  
**Title:** Glutamine Synthetase Inhibitors for Tuberculosis Therapy  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/07/01-2005/06/30

DESCRIPTION (Provided by the applicant:) Infection by *Mycobacterium tuberculosis* (MTB) is a significant complication experienced by many AIDS patients. Recently, the enzyme glutamine synthetase (GS) has become a new therapeutic target for MTB, due to the demonstration that secretion of enzymatically active MTB GS is required for survival and pathogenicity of the organism. The long-term goal of the proposed research is to develop inhibitors of MTB GS with potential therapeutic use. Many in vitro inhibitors of bacterial GS's have been documented, but none are useful clinically. In order to initiate MTB GS inhibitor design, a portion of this proposal is aimed at understanding its molecular properties, in comparison to the well studied *E. coli* GS. If comparable to the *E. coli* GS, then a new strategy will be pursued to obtain inhibitors that are more potent and selective than any previously described compounds. Specifically, the highly symmetrical ring structure of GS will be exploited to design a library of multivalent inhibitors which bind to the flexible loop on several subunits, in contrast to the monovalent inhibitors previously targeted individually to the active sites. The specific aims are: 1) To determine whether structural modification of the central loop on each subunit results in loss of enzyme activity. Because the modification of the central loop of the *E. coli* GS does lead to loss of activity, it is anticipated that this will be the case for MTB GS; 2) To design, synthesize and screen libraries of multivalent inhibitors targeted to the central loops of MTB GS, and to *E. coli* GS, for 'proof-of-principle.' Demonstration of the utility of multivalent inhibitors targeted to the central loops of MTB GS would provide a new rationale for GS inhibition and possibly for tuberculosis therapy.

**Grant:** 1R03AI051176-01  
**Program Director:** LAUGHON, BARBARA E.  
**Principal Investigator:** BARLETTA, RAUL G MS  
**Title:** Targeting M. tuberculosis alanine ligase for drug design  
**Institution:** UNIVERSITY OF NEBRASKA LINCOLN LINCOLN, NE  
**Project Period:** 2002/08/01-2004/07/31

DESCRIPTION (Provided by the applicant): Mycobacterium tuberculosis causes a serious chronic infection in human beings. M. tuberculosis, along with Mycobacterium avium,, is a major opportunistic pathogen of AIDS patients. Although generally susceptible to antimycobacterial agents, multi-drug resistant strains of M. tuberculosis have emerged, underlying the need for new therapeutic agents. Peptidoglycan is the backbone of the mycobacterial cell wall, and drugs that inhibit its biosynthesis cause a bactericidal effect due to cell lysis. D-alanine is a required component of the mycobacterial peptidoglycan. Thus, those biosynthetic enzymes involved in the synthesis and incorporation of D-alanine are attractive targets for new drug development, especially because these enzymes are not found in mammalian hosts. The terminal D-alanyl-D-alanine dipeptide of the peptidoglycan side chain is an essential component for this process and its synthesis is catalyzed by the enzyme D-alanyl-D- alanine synthetase, usually denominated D-alanine ligase (Ddl). Unfortunately, the specific characteristics of the M. tuberculosis enzyme have not been fully characterized, nor the essentiality of the gene has been elucidated. In this context, our hypothesis for the proposed project is that D-alanine ligase plays an essential role in M. tuberculosis physiology and is a useful target for drug design. To test this hypothesis, we plan to: 1) Overexpress, purify, and characterize biochemically the M. tuberculosis Ddl enzyme; and 2) Test the essential role of Ddl enzyme in M. tuberculosis physiology. These studies are expected to provide basic knowledge on key enzymes involved in the pathway of peptidoglycan biosynthesis in mycobacteria. Most importantly, we will obtain information on the physiological essentiality and biochemical parameters of the Ddl enzyme necessary to develop assays for the screening and testing of candidate compounds.

**Grant:** 1R03AI053074-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** ERNST, JOEL D  
**Title:** Tuberculosis Immunity: Essential Host Genes  
**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO  
SAN FRANCISCO, CA  
**Project Period:** 2002/09/15-2002/12/31

DESCRIPTION (provided by applicant): Despite improved tuberculosis control measures in the United States, tuberculosis continues to be a disease of growing worldwide importance. The currently-available drugs and vaccine (BCG) are efficacious, but are insufficient to prevent tuberculosis from continuing to spread and cause morbidity and mortality. An improved vaccine, developed and evaluated with better understanding of the mechanisms of protective immunity required for control of tuberculosis, has the theoretical potential of markedly improving prevention of tuberculosis. While it is clearly established that the cytokine, interferon gamma, is essential for protective immunity to tuberculosis, the mechanisms of interferon gamma in protective immunity are incompletely understood. In particular, the host genes whose expression is regulated by interferon gamma and that mediate protection against tuberculosis remain to be identified. While nitric oxide synthetase is one of these genes, there is strong evidence that additional interferon gamma-regulated genes contribute to control of tuberculosis. In this project, we propose experiments whose long term goal is to identify interferon gamma-regulated genes that are essential for immune control of tuberculosis. We will first characterize the course of M. tuberculosis infection in mice lacking each of three transcription factors (STAT1, IRF1, and ICSPB) that mediate the effects of interferon gamma on gene expression, to determine whether the interferon gamma-regulated genes that control M. tuberculosis in the lung are downstream of one or more of these transcription factors. In addition, we will prepare bone marrow chimeric mice, to determine whether macrophages are the only cells whose response to interferon gamma contributes to control of M. tuberculosis in the lungs. Finally, we will use high-density DNA microarrays to analyze gene expression in the lungs of M. tuberculosis-infected mice lacking each of the interferon gamma-responsive transcription factors, to determine the feasibility of this approach in identifying the genes that mediate interferon gamma-dependent protective immunity to tuberculosis. The studies proposed in this application will contribute to the knowledge of the molecular and cellular mechanisms of immunity to tuberculosis. In addition, they will determine the feasibility of analysis of gene expression in the lungs of specific strains of mice to ultimately identify genes that confer protection against tuberculosis.

**Grant:** 1R03AI053079-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** HASSETT, DANIEL J PHD  
**Title:** B.pseudomallei bioterrorism and quorum sensing  
**Institution:** UNIVERSITY OF CINCINNATI CINCINNATI, OH  
**Project Period:** 2002/09/15-2004/09/14

DESCRIPTION (provided by applicant): A bioterrorist attack, although once thought to be a remote possibility in the United States, is now a very real and terrifying threat to the lives of all Americans. A major area of concern is the distribution and survival of pathogenic bacteria in lakes, rivers, streams, swimming pools, our water supply and soils. *Burkholderia pseudomallei* (BP) is a Biosafety level 3 pathogen that is normally a saprophyte of soil and water in tropical areas of Southeast Asia, and causes a glanders-like disease called melioidosis. Melioidosis can exist as a benign pulmonary form, but may also develop into a rapidly fatal septicemia. If left untreated, >95% of all cases are fatal within 2 days of exposure. According to the US Department of Agriculture, glanders was eradicated from the US animal population in 1934. However, bioterrorists can easily bring such organisms into our country and distribute the organism into many bodies of water, water holding tanks and heavily populated areas where significant aerosols can be produced. Further complicating matters is the fact that bacteria growing as a biofilm are remarkably recalcitrant to biocides and antibiotics. The processes of quorum sensing (QS), a form of cell-cell communication, in the related organism *Pseudomonas aeruginosa*, is critical for biofilm formation, virulence and resistance to biocides. Because the BP genome harbors homologs to known QS genes, we hypothesize that it, too, participates in QS and uses it for optimal biofilm formation and resistance to biocides. These studies will provide important information that will lead to countermeasures against a potential bioterrorist attack using the potentially lethal gram-negative bacterium, *B. pseudomallei*.



**Grant:** 1R03AI053082-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** HALDAR, KASTURI PHD BIOCHEMISTRY, OTH  
**Title:** Cholesterol transport and Salmonella Pathogenesis  
**Institution:** NORTHWESTERN UNIVERSITY EVANSTON, IL  
**Project Period:** 2002/09/30-2004/09/29

DESCRIPTION (provided by applicant): Cholesterol is an important component of eukaryotic cell membranes. It has been shown to modify diverse cellular processes from membrane transport events to transcriptional regulation in cells that underlie both neurodegenerative and heart diseases. Elevated cholesterol is also known to promote microbial infections by HIV, mycobacteria, and malarial parasites. Yet the molecular determinants of pathogens that underlie cholesterol sequestration in the vacuole have been difficult to identify. *S. Typhimurium* is a gram-negative bacterium that causes significant human disease and is also highly amenable to genetic manipulation. Cholesterol accumulates in the intracellular vacuole and the long-term aim of this proposal is to exploit genetic methods available in *S. Typhimurium* to identify bacterial determinants that underlie both infection and pathogenesis. The study will contribute to our basic understanding of how *S. Typhimurium* alters cholesterol distribution in cells and new strategies for limiting bacterial proliferation in the Salmonella-containing vacuoles (SCV). Molecular, genetic tools including gene knock outs and gene screens, combined with high resolution imaging techniques and biochemical subcellular fractionation assays, will be used to identify bacterial genes that are responsible for acquisition and balance of cholesterol in the SCV. The consequence of ablation of bacterial gene products will be evaluated in an in vitro tissue culture model as well as in animals. Their homologues in other microbial pathogens as well as mammalian cells may reveal new pathogenic determinants and new mechanisms underlying cholesterol homeostasis.

**Grant:** 1R03AI053122-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** MENZIES, BARBARA E BA  
**Title:** STAPHYLOCOCCUS AUREUS FIBRONECTIN-BINDING ADHESINS  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/09/30-2004/09/29

DESCRIPTION (provided by applicant): Staphylococcus aureus continues to be a major human pathogen causing skin and deep soft-tissue infections that may complicate implanted devices and surgical wound sites. The increasing prevalence of staphylococcal strains that are resistant to antibiotics looms as a threat to patients and the public health. Novel preventive and therapeutic approaches are needed for combating this pathogen. Since adherence of staphylococci to host tissues is critical for colonization and infection, staphylococcal surface proteins are likely to be the first point of contact between the bacterium and the host and, thus, are important preventive targets. Of the staphylococcal surface proteins that bind to host structures, the fibronectin-binding proteins (FnBP) are the major fibronectin (Fn)-binding adhesions and mediate bacterial adherence and intracellular invasion in vitro. A recent study has confirmed the importance of FnBP in the initiation of infection in an endocarditis model. Unfortunately, most attempts to use FnBP as a vaccine target have been hampered by poor blocking capacity of anti-FnBP antibodies. In a non-vaccine approach, a fragment of the FnBP of S. aureus has been used to prevent the establishment of wound infection in a guinea pig model. One explanation of this mode of action of FnBP is that it competitively blocks the binding of staphylococci to host targets such as Fn and/or other receptors. The preventive effects of this FnBP fragment in wound infection prophylaxis are likely broadly applicable to most S. aureus strains given that the capacity to bind Fn is common for almost all staphylococcal strains. Observations from prior studies of the FnBP molecule using recombinant and synthetic FnBP constructs will be applied to the wound infection model in an effort to identify critical regions within FnBP that are responsible for the preventive effect in this animal model. Also, anti-adhesive and/or inflammatory mechanisms that may be operative in the preventive effect of FnBP will be examined. The use of FnBP as an anti-adhesin presents a novel therapeutic tool for the prevention of S. aureus infections.

**Grant:** 1R03AI053179-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** NARASIMHAN, SUKANYA PHD  
**Title:** Molecular analysis of tick-spirochete interactions  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2002/09/15-2004/09/14

DESCRIPTION (provided by the applicant): Ticks are significant vectors of disease to cattle and humans. The proposed project aims to describe the molecular interactions between the vector, *Ixodes scapularis* and the pathogen *Borrelia burgdorferi* (*B. burgdorferi*), the causative agent of Lyme disease. The gene expression profile of the tick salivary glands and midguts will be examined in the presence and absence of *B. burgdorferi* infection using a gel based differential display technique. The functions of the protein (s) encoded by the identified gene(s) will be inferred initially by computer based data mining. The functional significance of these gene products in *B. burgdorferi* development in the tick tissues will be explored in the presence of antibodies raised against candidate recombinant proteins. The results of this project will provide (a) an understanding of molecular mechanisms underlying the development of *B. burgdorferi* in the tick vector (b) provide insights into molecular manipulations of the vector in the context of *B. burgdorferi* infection and (c) provide clues to the function of differentially expressed spirochete and vector genes. The ultimate goal of this project is to identify novel targets based on both the vector and the pathogen for blocking transmission of Lyme disease. This investigation will also serve to pave the way for elucidation of mechanisms underlying transmission of other tick-borne pathogens.

**Grant:** 1R03AI053184-01  
**Program Director:** DUNCAN, RORY A.  
**Principal Investigator:** ASKEW, DAVID S PHD  
**Title:** A tetR/tetO-regulated promoter system for *A. fumigatus*  
**Institution:** UNIVERSITY OF CINCINNATI CINCINNATI, OH  
**Project Period:** 2002/09/15-2004/09/14

DESCRIPTION (provided by applicant): *Aspergillus fumigatus* is a major obstacle to the successful treatment of bone marrow and solid organ transplant recipients worldwide. The organism is a potent opportunistic fungal pathogen, causing severe invasive infections that result in mortality rates that approach 90 percent. The continued expansion of organ transplantation programs, and the lack of effective antifungal therapy to treat invasive aspergillosis, is driving the need for a more detailed understanding of the *A. fumigatus* genes that contribute to pathogenesis. Unfortunately, the genetic tractability of *A. fumigatus* has lagged behind most other fungal systems, which limits the type of experiments that can be performed on this organism. Inducible promoter systems are one of the most important tools in fungal genetics and have proven to be instrumental for the elucidation of gene function in a number of species. The purpose of this proposal is to develop the technology for a regulatable gene expression system in *A. fumigatus*, focusing on the prokaryotic tetR/tetO system that has been applied to other eukaryotes. The first aim of the project is to engineer the tetR/tetO system so that a gene can be switched off in *A. fumigatus* in the presence of tetracycline. This will be accomplished by creating a promoter containing one or more copies of the tet operator sequence linked to a minimal TATA-promoter, and using this hybrid promoter to drive expression of an *E. coli* lacZ reporter gene. The expression cassette will be transformed into strains of *A. fumigatus* that constitutively express an artificial transactivator comprised of the tet repressor tetR linked to the Herpes simplex VP16 activator domain, and the activity of the reporter gene will be quantitated in the presence or absence of tetracycline. The second aim seeks to determine whether this system can be used to manipulate *A. fumigatus* gene expression in vivo. Strains carrying the tet-regulated reporter system will be used for infection in a mouse model of invasive aspergillosis, and the expression of the reporter gene in mouse tissues will be determined in the presence and absence of tetracycline. The ability to manipulate gene expression in vivo would provide a unique opportunity to assess the contribution of a specific *A. fumigatus* gene product to the pathogenesis of aspergillosis.

**Grant:** 1R03AI053192-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** GLATMAN-FREEDMAN, AHARONA MD  
**Title:** Novel Approach for Vaccine Development against M.Tb  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2002/09/15-2004/09/14

DESCRIPTION (provided by applicant): The overall aim of this proposal is to develop vaccine candidates which will induce protective antibody response for the prevention of M. tuberculosis infections. In contrast to other vaccine strategies for tuberculosis, this strategy will rely on activating the humoral immune response. Studies from our group have shown that a murine monoclonal antibody (mAb) 9d8, which recognizes the mycobacterial polysaccharide arabinomannan, enhances survival in mice infected with M. tuberculosis. These results suggest that arabinomannan may be a biologically important target for the humoral immune system. Our experimental approach will be to identify additional protective monoclonal antibodies to arabinomannan, which will be used to isolate arabinomannan fractions rich in protective epitopes; conjugate arabinomannan to a protein carrier and demonstrate its ability to elicit a protective antibody response. The specific aims of this project are: 1) To generate and identify protective monoclonal antibodies to arabinomannan. 2) To isolate mycobacterial arabinomannan fractions and generate candidate vaccines. 3) To determine the protective efficacy of arabinomannan and arabinomannan-protein conjugate vaccine candidates against Mtb infection.

**Grant:** 1R03AI053202-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** HAIMOVICH, BEATRICE PHD  
**Title:** Apoptotic and non-apoptotic death responses to Shigella  
**Institution:** UNIV OF MED/DENT NJ-R W JOHNSON MED PISCATAWAY, NJ  
SCH  
**Project Period:** 2002/09/17-2004/09/16

DESCRIPTION (provided by applicant): Shigella is an enteric pathogen that causes dysentery in humans. The pathogen is most often acquired by ingestion of contaminated food or water. The annual number of Shigella episodes throughout the world is thought to exceed 150 million. Greater than 90% of the cases occur in developing third world countries. The number of deaths attributed to Shigella exceeds one million per year. The majority of the casualties (>65%) are children under the age of five. Although the mechanism by which Shigella species cause the disease is still not fully understood, the strong inflammatory reaction characteristic to Shigellosis provides a strong indication that host inflammatory cells play a key role relative to the onset of the disease. We hypothesize that Shigella has developed strategies to kills host macrophages and neutrophils in order to survive its first encounter with the host immune surveillance system. The overall goal of studies described in this application is to develop an understanding of the mechanism by which Shigella kills host, human macrophage. Data obtained by the applicants strongly argue that Shigella triggers a non-apoptotic death of human macrophages while Shigella-infected human monocytes die by an accelerated apoptotic process. The studies proposed will test the hypotheses that Shigella-infected macrophages die by a necrotic/non-apoptotic mechanism that i) results from the production of reactive oxygen intermediates that cause the destruction of the mitochondria and/or ii) that a protein produced by virulent Shigella specifically targets the host-cell's mitochondria for destruction. The studies proposed will address two specific aims. Aim 1: Is the non-apoptotic death of human macrophages triggered by virulent Shigella linked to, and dependent on, the production of reactive oxygen intermediates by the infected cells? Aim 2: Is the response of human macrophages to Shigella dependent on the Shigella protein IpgB1?

**Grant:** 1R03AI053214-01  
**Program Director:** SAWYER, LEIGH A.  
**Principal Investigator:** EMCH, MICHAEL E PHD  
**Title:** Geographical Analysis in Vaccine Efficacy Trials  
**Institution:** PORTLAND STATE UNIVERSITY PORTLAND, OR  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): This study will develop and test spatial analytical methods for vaccine trials to control for spatially heterogeneous disease exposures (spatial effect modifiers) and spatial bias in disease outcomes (spatial confounders). In 1985, a community-based individually randomized oral cholera vaccine trial was conducted in Matlab, Bangladesh. The double-blind trial measured the efficacy of two vaccines, the B subunit-killed whole cell (BS-WC) and the killed whole cell only (WC) vaccine. Women over 15 and children aged 2 to 15 were the target group in the trial. To identify the cholera cases from the study area, the investigators conducted a passive surveillance at one hospital and two community-based treatment centers. Passive surveillance can introduce bias because access to treatment centers, which is usually a function of distance, influences health-seeking behavior. Also, efficacy might differ in different parts of the study area because socio-environmental circumstances and therefore disease exposure levels vary in space. This study will use a geographic information system (GIS) to determine: (1) how cholera vaccine efficacy varies spatially in the study area; (2) what ecological socio-environmental variables are related to cholera vaccine efficacy (i.e., which variables are effect modifiers); (3) how protective efficacy varies with access to treatment facilities (i.e., whether access is a spatial confounder); and (4) whether cholera incidence in the placebo group is related to vaccine coverage rates (i.e., is herd immunity important?). The study will use three data sets, previously collected by the investigators, including: (1) a large cholera vaccine trial database, (2) a comprehensive longitudinal demographic database of the rural population of approximately 200,000 from which the vaccine trial participants were selected, and (3) an accurate household-level spatial database of the same study area population. The results of this project can help with the design of all future vaccine trials.

**Grant:** 1R03AI053250-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** FREDRICKS, DAVID N  
**Title:** The Molecular Microbiology of Bacterial Vaginosis  
**Institution:** FRED HUTCHINSON CANCER RESEARCH SEATTLE, WA  
CENTER  
**Project Period:** 2002/09/20-2004/08/31

DESCRIPTION (provided by applicant): Bacterial vaginosis (BV) is a condition that affects millions of women and is linked to several serious health conditions, including preterm labor, cervical intraepithelial neoplasia, and HIV infection. About half of women with BV complain of a malodorous vaginal discharge, and half are asymptomatic. The cause of BV is not known, though current evidence suggests that women with BV undergo a change in the bacterial flora of the vagina. No single cultivated bacterium has been definitively determined to cause BV. Advanced methods in molecular biology have recently been used to study environmental and human ecosystems, allowing investigators to detect and identify microbes without cultivation. These studies reveal many novel, cultivation-resistant bacteria, and expand our understanding of the microbial diversity in these niches. We propose to apply the same molecular methods to the microbial ecosystem of the human vagina. The Specific Aims are to: 1. Create a census of the bacteria that inhabit the normal vagina. Vaginal fluid samples from 4 women without BV will be obtained and subjected to broad range PCR to directly amplify bacterial 16S rDNA without cultivation. The PCR products will be cloned into E. coli, and the clones screened by performing PCR on the inserts. Inserts of the correct size will be analyzed with PCR RFLP analysis, and unique inserts will be sequenced. Phylogenetic analysis of these 16S rDNA sequences will allow us to identify bacteria, or to infer evolutionary relationships for novel bacteria; 2. Create a census of the bacteria that inhabit the vagina of women with BV. Vaginal fluid samples from 4 women with BV will be subjected to the analysis outlined in aim 1; 3. Identify bacteria or bacterial communities that may be the cause of BV. Bacteria that are only associated with BV in our initial cohort will be selected for further study. Specific PCR assays will be developed and validated for each candidate pathogen. In future studies, vaginal fluid samples from a larger cohort of women will be assayed to determine if a candidate pathogen is specifically associated with BV.



**Grant:** 1R03AI053598-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** ZENG, MINGTAO PHD  
**Title:** Multi-component and easily administrated anthrax vaccine  
**Institution:** UNIVERSITY OF ROCHESTER ROCHESTER, NY  
**Project Period:** 2002/09/15-2004/09/14

DESCRIPTION (provided by applicant): Anthrax, which is caused by the spore-forming bacterium *Bacillus anthracis*, has become one of the major bioterrorism threats to our nation. Human vaccination in the USA with licensed protective antigen (PA)-based vaccine, Anthrax Vaccine Adsorbed (AVA), requires six immunizations followed by annual boosters. This underscores the need for development of new, improved anthrax vaccine. The long-term goal of this research is to develop an effective and easily administrated anthrax vaccine, using the B. anthracis protective antigen (PA63), the N-terminal domains of lethal factor (LFn, aminoacids 1-254) and edema factor (EFn, aminoacids 1-254) as vaccine components. Our hypothesis is that an effective vaccine should be composed of multiple relevant antigens delivered by an intranasal route in order to provide mucosal and systemic immunity against anthrax. Formulation of the anthrax vaccine into a nasal spray would allow a mass population to be immunized in a short period at a low cost. Among the currently available mucosal immunization strategies, antigen delivery with a replication-defective adenovirus is a good choice. Recombinant adenovirus and plasmid expression vectors encoding PA63, LFn and EFn will be constructed through this project. In order to evaluate the efficacy of the vaccine and provide an optimal vaccination protocol, intranasal immunization with different combinations of adenoviral vectors will be compared with immunization with plasmid expression vectors by intramuscular injection. The specific aims of this project are: Specific Aim #1: To develop a recombinant adenovirus-vectored multi-component vaccine against anthrax. Specific Aim #2: To compare the systemic and mucosal immunity elicited by the vaccine developed in #1 through intranasal inoculation with that elicited by plasmid expression vectors through intramuscular injection.

**Grant:** 1R03AI053700-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** KUDVA, INDIRA T PHD  
**Title:** Signature profiling of HUS-causing E. coli O157:H7  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2002/09/15-2004/09/14

DESCRIPTION (provided by applicant): The objective of this proposal is to identify genes that are unique to E. coli O157:H7 (E. coli O157) isolates capable of causing hemolytic uremic syndrome (HUS) in humans, and develop a signature profiling system for such isolates. Toward this objective, a HUS-causing E. coli O157 isolate will be compared to a bovine E. coli O157 isolate from lineage II that is incapable of human infection. Convalescent sera from patients who had HUS will be absorbed with a library made from the genomic DNA of the bovine E. coli O157 isolate, and then used to screen a library made from the genomic DNA of the HUS-causing E. coli O157 isolate. Genes unique to the HUS-causing isolate will be identified and PCR primer pairs derived from their DNA sequences will be used to establish a signature profiling system. All of these signature profiling primer pairs will then be evaluated against a collection of O157 isolates from different sources and disease outcomes. The proposed immunological approach will provide a more direct, powerful and relevant-to-infection approach to the detection of genes unique to the HUS-causing isolates. We anticipate that this research will facilitate the development of a signature profiling system to help prognosticate human disease outcome, aid in the development of specific therapeutic measures, and improve epidemiological surveillance.

**Grant:** 1R03AI053754-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** RILEY, LEE W  
**Title:** Epidemiology of an uropathogenic E coli clonal group  
**Institution:** UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA  
**Project Period:** 2002/09/20-2004/09/19

DESCRIPTION (provided by applicant): This is a pilot project that aims to characterize the epidemiology of a recently identified clonal group of uropathogenic *Escherichia coli* designated as CgA. CgA refers to a set of multidrug-resistant *E. coli* strains that have identical ERIC PCR patterns and identical or similar pulsed field gel electrophoresis (PFGE) patterns, that belong to serotypes O11:nt and O77:nt, and that share identical or similar virulence factor profiles. In a multicenter study, we discovered that CgA accounted for a substantial proportion of community-acquired drug-resistant urinary tract infections (UTI) in 3 distinct university communities in the United States. The widespread dissemination of CgA raised the possibility that this clonal group of *E. coli* may be spread by contaminated ingestible vehicles. We hypothesize that the increase in prevalence of drug-resistant UTI in a community is affected not only by the frequency in the use of antimicrobial agents, but also by the introduction into such communities of clonal groups of drug-resistant uropathogenic *E. coli* by contaminated vehicles. In this application, we wish to: (1) identify the geographic distribution of CgA and the clinical spectrum of infections caused by this organism, including complications of UTI; and (2) reservoir and risk factors for infection with CgA. The geographic distribution of CgA will be studied by the analyses of *E. coli* archives obtained by the PI from northern California, Brazil, and New York, and by the collaborator (Dr. James Johnson) from Minnesota, Iowa, Washington, and a WHO Reference Laboratory. The incidence and prevalence of complications of UTI (pyelonephritis and bacteremia) caused by CgA will be determined by the analyses of *E. coli* isolates obtained from patients in a San Francisco hospital in collaboration with Dr. Francoise Perdreau-Remington. The possible animal reservoir for CgA will be examined by the analysis of all available O11:nt and O77:nt *E. coli* isolates from animals in collaboration with Dr. Chobi DebRoy. Through this pilot study, we wish to provide preliminary evidence in support of the hypothesis that drug-resistant uropathogenic *E. coli* can be spread by contaminated ingestible vehicles, and use the data generated from this project to design long-term studies to better characterize risk factors associated with CgA infection.

**Grant:** 2R15AI043317-02  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** CASTRIC, PETER A  
**Title:** Studies on the glycosylation of *P. aeruginosa* pili  
**Institution:** DUQUESNE UNIVERSITY PITTSBURGH, PA  
**Project Period:** 1998/07/15-2003/02/28

DESCRIPTION (provided by the applicant): *Pseudomonas aeruginosa* persists as a major cause of life-threatening infections for individuals with the following conditions: cystic fibrosis, burns, wounds, cancer (leukemias), those receiving immunosuppressive therapy, diabetics, as well as intravenous drug users. The pili of this bacterium are protein filaments that extend from the ends of the cell. These structures serve as adhesion factors, and so are important virulence factors. The long-term objectives of this project are to determine the role of pilus glycosylation in *P. aeruginosa* pathogenesis and to ascertain the importance of the glycan in pilus vaccine design. Work from my laboratory has shown that the pili of *P. aeruginosa* 1244 are glycosylated with a trisaccharide moiety originating in the lipopolysaccharide O-antigen biosynthetic pathway. The research in this proposal aims to determine the structural relationship between the pilin glycan and the O-antigen. In addition, it will explore the ability of the glycosylation system to attach heterologous glycan to pilin. The project will determine the glycan precursor involved in pilin glycosylation and elucidate the point in pilus biogenesis where glycosylation occurs. The project will determine the step in O-antigen biosynthesis that produces the glycan and the subcellular location of pilin glycosylation.

**Grant:** 2R15AI047165-02  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** BLAZYK, JOHN F PHD  
**Title:** Design of Novel Linear Cationic Antimicrobial Peptides  
**Institution:** OHIO UNIVERSITY ATHENS ATHENS, OH  
**Project Period:** 2000/06/01-2005/06/30

DESCRIPTION (provided by applicant): Widespread resistance to antibiotics in current clinical use is increasing at an alarming rate. Novel approaches in antimicrobial therapy will be required in the near future to maintain control of infectious diseases. An enormous array of small cationic peptides exists in nature as part of the innate defense systems of organisms ranging from bacteria to humans. For most linear peptides, such as magainins and cecropins, a common feature is their capacity to form an amphipathic alpha-helix (with polar and nonpolar groups on opposite faces of the helix), a structural feature believed to be important in their antimicrobial function as membrane-lytic agents. A massive effort over the past ten years has resulted in a better understanding of the molecular mechanism of these antimicrobial peptides and the production of more potent analogues. To date, however, few of these peptides appear to have clinical potential, especially for systemic use, due to insufficient selectivity between target and host cells. Recently we developed a new strategy in the design of antimicrobial peptides. These linear cationic peptides, which form amphipathic beta-sheets rather than alpha-helices, demonstrated superior selectivity in binding to the lipids contained in bacterial vs. mammalian plasma membranes. We propose here to extend the investigation of this new class of peptides by studying smaller compounds of similar design to: 1) define the minimum peptide length necessary for antimicrobial activity; 2) probe the sequence dependence for maximal activity and selectivity by using strategic tryptophan, alanine and glycine substitutions; 3) explore the mode of action and compare the molecular mechanism to that of other antimicrobial peptides; 4) measure peptide activity and stability under physiological conditions; and 5) test for cytotoxicity on normal and transformed human cells. This project should result in the design of smaller and more selective antimicrobial peptides that produces leads for animal and clinical testing. As the arsenal of available antibiotics dwindles due to ever-increasing resistance of bacteria to the drugs in clinical use, these linear beta-sheet-forming antimicrobial peptides provide a new avenue worthy of further exploration.

**Grant:** 1R15AI047801-01A2  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** SCHWAN, WILLIAM R PHD  
**Title:** Proline uptake in staphylococcus aureus pathogenesis  
**Institution:** UNIVERSITY OF WISCONSIN LA CROSSE LA CROSSE, WI  
**Project Period:** 2002/05/01-2006/01/31

Staphylococcus aureus is a significant human pathogen, which is the leading cause of surgical-wound infections and the second most frequent cause of nosocomial bloodstream infections in the United States. A significant number of cases of food poisoning are also linked to contamination of foodstuffs with *S. aureus*. Almost every tissue and organ within the human body is susceptible to infections by this species. Many of the current infections are caused by staphylococcal strains that are resistant to one or more antibiotics. Eighty to ninety percent of all *S. aureus* strains are resistant to the antibiotic penicillin and up to fifty percent of all strains isolated from patients in hospitals are resistant to methicillin. Recent outbreaks of community-acquired *S. aureus* possessing methicillin resistance and the emergence of vancomycin-resistant *S. aureus* strains mean that some strains may be untreatable by any antibiotic. Because *S. aureus* is able to infect so many different tissues within the human body, this grant proposes to study proline transport in *S. aureus* as a means to study the role proline transporters play in the pathogenesis of the bacteria. At least two proline transport systems are known for *S. aureus*. This grant proposes to identify the homolog of the ProP low affinity proline transporter and mutate the *prop* gene by allelic exchange or transposon mutagenesis with Tn917. With this *proP* mutant, a *proPputP* double mutant will then be created. Both the single and double mutants will be tested for proline transport differences in vitro and for their attenuation in animal models of infection. The regulation of the high affinity proline transport gene, *putP*, also will be tested in vitro in proline uptake assays and in vivo in several animal models of infection using a *putP* transcriptional fusion. The results of this study will help us understand the role of proline transport in *S. aureus* infections.

**Grant:** 1R15AI050638-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** MCLEAN, ROBERT J PHD ENVIRONMENTAL  
MICROBIOLOGY  
**Title:** Transcriptional Profiles of Escherichia coli Biofilms  
**Institution:** SOUTHWEST TEXAS STATE UNIVERSITY SAN MARCOS, TX  
**Project Period:** 2002/04/15-2005/04/14

**DESCRIPTION** (provided by the applicant): The majority of bacterial infections (65 percent) involve growth of the organisms as surface-adherent, biofilms. Within biofilms bacteria are protected from a number of stresses such as antimicrobial agents, nutrient fluctuation, and host defenses. While a number of studies have identified genes involved in the early stages of biofilm formation, very little is known about the physiology and gene expression of established biofilms. The present study will employ gene array technology to identify the genes that are being expressed in young, mature, and aged E. coli biofilms as previous studies have shown that antibiotic resistance increases with biofilm age. This is an exciting concept in that we can simultaneously determine and compare the use (expression levels) of all 4290 genes in E. coli during growth as attached (biofilm) and free-swimming (planktonic) forms. During the first part of this project, we will grow biofilms and planktonic E. coli at two different growth rates, using a continuous culture device (chemostat). In this fashion, we will be able to control the growth rate of the biofilm-forming bacteria. At various times, RNA will be extracted from the biofilm and planktonic populations, purified and used to produce labeled cDNA that can then be bound (hybridized) to a commercially available gene array. Analysis of the binding intensity will give an indication of the use (expression) of each gene. In the second part of this project, we will use this approach to investigate differences in gene expression in E. coli that lack the ability to grow slowly or survive starvation. Both of these characteristics (slow-growth and starvation survival) are considered to be necessary for biofilm formation. Overall, this project will explain several fundamental aspects of E. coli biofilm growth and suggest possible targets for future antibiotic research.

**Grant:** 1R15AI052101-01  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** MINNICK, MICHAEL F PHD  
**Title:** Bartonella Inhibitory Factor for Endothelial Cell Growth  
**Institution:** UNIVERSITY OF MONTANA MISSOULA, MT  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Five species of Bartonella are emerging agents of infectious human disease. Because of their ability to parasitize erythrocytes and endothelial cells within the circulatory system, bartonellosis present with a wide array of cardiovascular manifestations including endocarditis, bacillary angiomatosis, peliosis, chronic bacteremia, and hemolytic anemia. Bartonella's parasitism of microvascular endothelial cells is characterized by a proliferative response triggered by a bacterial protein that we have termed Bartonella angiogenic protein (BAP). The resulting angiogenesis generates a pseudoneoplastic vascular lesion. In our work on BAP, we recently discovered an inhibitory protein from Bartonella henselae that significantly reduces the growth of human vascular endothelial cells, termed BIF. Production of BAP and BIF by Bartonella suggests that the pathogen plays an active role in regulating the growth of its host cell. However, neither the molecular nature nor mechanism of BAP or BIF are known. Therefore, the long-term goals of this project are to characterize BIF and its mechanism of action, using Bartonella henselae as a model for the genus. Specific goals to achieve these objectives will: 1) Characterize BIF at the molecular level using biochemical and molecular biological techniques, 2) Analyze BIF's mechanism by mapping domains necessary to its inhibitory activity by creating overlapping BAP deletion mutants and assaying their ability to inhibit vascular endothelial cell growth, 3) Examine BIF and BAP for antagonism in vitro and assess the angiostatic activity of BIF in vivo, and 4) initiate studies to elucidate BIF's mechanism by identifying and characterizing its cognate endothelial cell receptor(s). These experiments will generate valuable data on the molecular nature of a novel bacterial protein that can modulate the growth of human vascular endothelial cells and will help elucidate the mechanism whereby angiomatous and pseudoneoplastic disease manifestations of bartonellosis are generated during infection.



**Grant:** 1R15AI054382-01  
**Program Director:** MILLER, MARISSA A.  
**Principal Investigator:** GUSTAFSON, JOHN E PHD  
**Title:** Novel antibiotic resistance mechanisms in *S. aureus*  
**Institution:** NEW MEXICO STATE UNIVERSITY LAS LAS CRUCES, NM  
CRUCES  
**Project Period:** 2002/07/15-2005/07/14

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is responsible for staggering mortality and financial loss in the U.S. We have characterized a salicylate-inducible multidrug resistance mechanism in *S. aureus*. Salicylate-induced multidrug resistance is expressed phenotypically and involves reduced drug accumulation. Previously intrinsic multidrug resistance in *S. aureus* was attributed to the multidrug efflux pump NorA. We provide evidence that demonstrates that the salicylate-induced multidrug resistance mechanism is NorA-independent. We will also characterize a genotypic *S. aureus* multidrug resistant mechanism that results as a consequence of selection for resistance to the antimicrobial terpene mixture pinesol. This mechanism also increases resistance to the cell wall active antistaphylococcal antibiotic vancomycin. We intend to determine if the putative *S. aureus* multidrug efflux pump AcrB and/or the alternative transcription factor sigma B play roles in the multidrug resistance mechanisms of *S. aureus* being studied in our laboratories. We also plan to demonstrate that pinesol-selected multidrug resistant mutants and salicylate treated cells express altered cell wall physiology. Genes involved with pinesol-selected and salicylate-inducible multidrug resistance mechanisms of *S. aureus* will be identified using two procedures: Two-dimensional gel electrophoresis and a DNA subarray constructed with genes suspected to be associated with multidrug resistance in *S. aureus*. We hypothesize that multidrug resistance expression by *S. aureus* will involve a large collection of genes and gene products. We also hypothesize that the putative multidrug efflux pump AcrB and alternative transcription factor sigma B of *S. aureus* will play a role in the multidrug resistance expression of: i.) normal *S. aureus* cells; ii.) pinesol-selected mutants of *S. aureus*; and iii.) salicylate-induced *S. aureus* cells. Novel components associated with intrinsic multidrug resistance mechanisms in *S. aureus* might be used as targets in the development of antimicrobials used to either prevent antibiotic resistant *S. aureus* from arising or to halt the growth of *S. aureus* involved with active disease. This research will also contribute to the growing body of knowledge on the interaction of the Gram-positive coccal cells with biocides and antibiotics.

**Grant:** 2R21AI020723-19A1

**Program Director:** RUBIN, FRAN A.

**Principal Investigator:** SCOTT, JUNE R

PHD

MICROBIOLOGY:BACTERIOLOGY

OGY

**Title:** Genetic Analysis of S Pyogenes Virulence Factors

**Institution:** EMORY UNIVERSITY

ATLANTA, GA

**Project Period:** 1984/06/01-2004/08/31

DESCRIPTION (provided by applicant): Streptococcus pyogenes, the group A streptococcus (GAS) is an important human pathogen causing frequent self-limiting diseases which may lead to serious sequelae. In addition, the GAS seems to be "re-emerging" as an important pathogen which causes life-threatening invasive disease. We will continue to use molecular genetic approaches to obtain a better understanding of known and presumed virulence factors of GAS and to identify new ones and to elucidate their regulation.

**Grant:** 2R21AI023348-14A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** LOW, DAVID A  
**Title:** DNA Methylation and Pili Gene Regulation  
**Institution:** UNIVERSITY OF CALIFORNIA SANTA BARBARA, CA  
BARBARA  
**Project Period:** 1987/04/01-2004/04/14

**DESCRIPTION:** (provided by applicant): The overall objective of this project is to understand the mechanisms by which DNA adenine methylase (DAM), leucine-responsive regulatory protein (Lrp), and PapI orchestrate the reversible switch between OFF and ON Pap pili expression states in uropathogenic *Escherichia coli* (UPEC). Since Pap pili are an essential virulence determinant of UPEC, this work has direct application to addressing the problem of urinary tract infections. PapI is required for translocation of Lrp between promoter-proximal pap sites 1-2-3 and distal sites 4-5-6. The methylation status of GATC sites within the central pap sites 2 and 5 controls binding of Lrp and PapI-Lrp. Binding of Lrp to promoter proximal sites represses pap transcription whereas binding of Lrp to distal sites initiates activation of pap transcription. The first aim of this proposal is to determine how DAM and PapI control binding of Lrp to sites 1-2-3 and 4-5-6. The hypothesis that PapI alters Lrp binding specificity, facilitating interaction with specific base-pairs present within sites 2 and 5 will be tested. The base-pair contacts between PapI-Lrp and sites 2 and 5 will be identified by missing contact, SELEX, and mutational analyses. The second aim is to identify amino acids of Lrp that play important roles in responsiveness to PapI and DNA methylation. This will be accomplished by isolation of lrp mutants with altered responses to PapI and DNA methylation and by a genetic suppressor approach using pap mutants isolated in Aim 1. Photo-crosslinking studies are proposed to directly identify amino acids within Lrp that interact with sites 2 and 5, and to determine how these interactions are altered by GATC site methylation. The third aim focuses on in vivo Pap switch dynamics. The hypothesis that DNA replication is required for Pap pili switching will be tested by monitoring pap pili gene expression by fluorescence activated cell sorting in synchronized cells following induction of PapI. Further analysis of the methylation states of the pap GATC sites following DNA replication will be done to determine the roles of DNA methylation in phase switching. Lastly, studies are proposed to determine how the phase switch is controlled in UPEC under normal physiologic conditions. The studies proposed here will provide a framework for understanding how DNA methylation patterns regulate pili gene expression since many other pili operons expressed by pathogenic bacteria share common regulatory features with pap. This work has wide clinical relevance since DAM is essential for the virulence of a number of pathogens including UPEC, *Salmonella typhimurium*, and *Yersinia pseudotuberculosis*. Finally, the pap system is a paradigm for the study of epigenetic regulation in eukaryotes, in which DNA methylation regulates biological processes including imprinting, tumor formation, and gene silencing.

**Grant:** 2R21AI037139-07  
**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:** 1996/06/01-2004/07/31

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 new antibiotics. The cell wall of its causative agent, *Mycobacterium tuberculosis*, is known to be a target of some of the most effective antimycobacterial drugs including ethambutol which has been known to inhibit the biosynthesis of arabinan of the cell wall proper and associated lipoarabinomannan (LAM). A diverse range of biological studies over a decade has collectively provided compelling evidence implicating LAM as a key surface molecule in host-pathogen interactions. The finding of immature LAM or truncated LAM as a consequence of natural and laboratory induced resistance to ethambutol, and embC gene knockout events provide invaluable model compounds for both structural and functional studies aiming at defining the relevance of LAM in pathogenesis. Specifically, with the availability of new enzymes, advanced chemical, NrvIR and mass spectrometric tools can be combined to yield the fine details of the arabinan assembly and the mode and site(s) of arabinan attachment to the mannan core. Enzymatically modified structural arabinan motifs positively correlating with particular biological attributes of clinical isolates will be derived from LAM, and neoarabinolipids will be generated for functional studies. Efforts will be given in resolving the heterogeneity in LAM and relate it to biology. CD1 restricted recognition of LAM by T cells will be examined in the context of cell mediated immunity in tuberculosis and its concomitant induction of cytokine secretion. Finally, gene knock-out mutants, cell-free assays and synthetic arabinofuranosyl acceptors will be utilized to establish the metabolic events involved in the arabinan assembly of LAM about which almost nothing is known, followed by identification of proteins (transferases) involved. Thus, the unifying theme of this Research Proposal is the structural analysis and manipulation of LAM, supplemented by genetic and biosynthetic studies leading to a better understanding of its biology and biosynthesis.

**Grant:** 2R21AI040689-06  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** SAHNI, SANJEEV K PHD  
**Title:** Rickettsia-Induced Transcriptional Activation  
**Institution:** UNIVERSITY OF ROCHESTER ROCHESTER, NY  
**Project Period:** 1997/08/01-2004/08/31

DESCRIPTION (provided by applicant): Rocky Mountain spotted fever, the most prevalent and important of the rickettsioses in the United States, is an acute tick-borne febrile illness caused by *Rickettsia rickettsii*. The organism infects and proliferates predominantly within vascular endothelial cells, which respond by activating a series of defense mechanisms, possibly via distinct signal transduction pathways. We have demonstrated that *R. rickettsii* infection of endothelial cells results in the activation of nuclear factor-kappaB (NF-kB a transcription factor which controls the expression of an array of genes involved in bacterial infections, immune response, and apoptosis. We have also shown that anti-apoptotic functions of NF-kB protect the host cell from apoptotic death during *R. rickettsii* infection. The objective of this application is to further our understanding of signaling mechanisms underlying Rickettsia-induced transcriptional activation and investigate their participation in the host cell response to infection. Since the seminal event in the activation of NF-kB is the degradation of Ikb (inhibitors of NF-kB) proteins by Ikb kinase (IKK) complex, Aim 1 will characterize the activation of IKK and phosphorylation/degradation of Ikb proteins during infection. We will determine the kinetics of activation of catalytic subunits, IKK-a and IKK-b, by an immunoprecipitation (IP) kinase assay. The role of the regulatory subunit, IKK-g will be evaluated using a specific, cell permeable peptide that blocks its association with the IKK complex. Aim 2 will characterize the activation of mitogen activated protein (MAP) kinases and investigate their involvement in rickettsial invasion of endothelial cells and activation of NF-kB Modulation of MAP kinase cascades, ERK1/2 and p38, will be examined by immunoblotting using phosphorylation state specific antibodies and activity assays by IP western analysis. Aim 3 will define the regulation of chemokine induction in response to infection and explore its dependence on the MAP kinase and NF-kB pathways. Using molecular biology and microscopy techniques and strains of *Rickettsia* with varying pathogenicity, we will investigate the correlation between infection, activation of IKK/NF-kB and MAP kinases, and induction of chemokine response. These studies will offer important perspectives in understanding rickettsial pathogenesis and provide valuable insight into the complex interplay of signaling events, which occur as part of the host-parasite relationship.

**Grant:** 2R21AI043063-05  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** CABELLO, FELIPE C MD OTHER AREAS  
**Title:** Stringent response and bmp expression in *B.burgdorferi*  
**Institution:** NEW YORK MEDICAL COLLEGE VALHALLA, NY  
**Project Period:** 1998/07/01-2003/08/31

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

**Grant:** 1R21AI048703-01A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** STEIN, DANIEL C PHD  
**Title:** Function of Opa and LOS in gonococcal pathogenesis  
**Institution:** UNIVERSITY OF MARYLAND COLLEGE PK COLLEGE PARK, MD  
CAMPUS  
**Project Period:** 2002/07/01-2004/06/30

DESCRIPTION (provided by applicant): *Neisseria gonorrhoeae* causes about 600,000 new infections each year in the United States, with health-care costs approaching 2 billion dollars/year. The costs and human suffering are amplified by the fact that concomitant gonococcal infections appear to facilitate HIV transmission. Gonococci preferentially infect the human urogenital tract, and its ability to enter and transcytose this mucosal surface is a chief cause of pelvic inflammatory disease, tubal infertility, ectopic pregnancy, and chronic pelvic pain. Various surface components, i.e., lipooligosaccharide (LOS) and opacity proteins (Opa), are important in mediating these diseases. Most studies have used tissue culture models to study the role of individual surface components in the pathogenic process, even though it is likely that multiple components are needed and/or are able to act synergistically. The fact that this pathogen manipulates its outer membrane suggests that such modifications are important in pathogenesis. Nothing is known about possible interactions between the surface molecules, because to date, we have lacked bacterial strains that have invariant, defined surfaces. This has prevented us from developing a comprehensive understanding of the pathogenesis of gonococcal disease. The objective of this proposal is to understand how gonococcal opacity proteins and lipooligosaccharides interact in the pathogenic process. The central hypothesis of the proposed research is that different variants of these surface antigens are important for the various stages of infection and/or symptom elicitation. We intend to test our hypotheses by pursuing three specific aims: We will determine how various surface structures interact in the invasion process, we will identify which receptors are responsible for binding specific LOS molecules and we will define the signal transduction pathways that are activated in host cells that are initiated by gonococcal adherence and/or invasion. The proposed research addresses the fundamental problem of how LOS and Opa interact in the disease process. We will define how LOS and Opa variation contribute to disease pathogenesis. The impact on human health is expected to be significant, because the new knowledge will likely make possible new approaches to the prevention and treatment of gonorrhea.

**Grant:** 1R21AI049322-01A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** ST. GEME, JOSEPH W MD  
**Title:** Haemophilus Hap-mediated Microcolony Formation  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2002/09/15-2003/09/14

DESCRIPTION (provided by applicant): Nontypable *Haemophilus influenzae* is a common cause of localized respiratory tract disease, especially otitis media, sinusitis, bronchitis, and pneumonia. In addition, this organism causes serious systemic disease, such as meningitis, endocarditis, and septicemia. The initial step in the pathogenesis of nontypable *H. influenzae* disease involves colonization of the upper respiratory mucosa. In studies with cultured epithelial cells, we have identified an *H. influenzae* serine protease called Hap, which facilitates intimate interaction with the epithelial surface and also promotes bacterial aggregation and microcolony formation. Based on our in vitro results, we speculate that Hap plays an important role in the process of colonization. Hap shares significant homology with the *H. influenzae* and *Neisseria gonorrhoeae* IgA1 proteases and belongs to the growing family of autotransporter proteins elaborated by pathogenic gram-negative bacteria. Like other members of this family, Hap is synthesized as a precursor protein with 3 functional domains, including an N-terminal signal sequence, an internal protease domain with adhesive activity (Hap-s), and a C-terminal outer membrane domain with translocator activity (Hap-beta). Ultimately, Hap undergoes autoproteolytic cleavage, with extracellular release of Hap-s. In recent work, we demonstrated that Hap-mediated adherence and microcolony formation are potentiated by a host protein called secretory leukocyte protease inhibitor (SLPI). This protein is present in respiratory secretions and inhibits Hap autoproteolysis, resulting in accumulation of surface-associated Hap-s. In the present proposal, we will focus on Hap-mediated adherence and microcolony formation. In Aim 1, we will solve the crystal structure of Hap-s and define the interactive surfaces involved in adherence and microcolony formation. In Aim 2, we will examine the ability of microcolonies to resist lactoferrin protease activity, to evade macrophage phagocytosis, and to enhance persistence in the chinchilla otitis media model. In Aim 3, we will characterize the relationship between respiratory viral infection and Hap-mediated adherence and microcolony formation, concentrating on the role of SLPI. From a practical perspective, the proposed studies may facilitate efforts to develop novel strategies for the treatment and prevention of *H. influenzae* disease. Perhaps more importantly, they may provide general insights into host-microbe relationships and expand our understanding of microbial biofilms.



**Grant:** 1R21AI049348-01A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** MECSAS, JOAN C BA  
**Title:** Genetics of Survival of *Yersinia pseudotuberculosis*  
**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA  
**Project Period:** 2002/05/01-2004/04/30

DESCRIPTION (provided by the applicant): *Yersinia pseudotuberculosis* is a gram-negative, enteric bacterial pathogen that causes gastroenteritis, mesenteric lymphadenitis, which can be misdiagnosed as appendicitis leading to unnecessary surgery, and occasionally systemic disease in humans and other mammals. To cause these syndromes, the bacteria colonizes a variety of tissues in a mammal, including the lumen throughout the gastrointestinal tract (GI), the Peyer patches, mesenteric lymph nodes, and eventually the spleen and liver. Colonization of each of these tissues requires expression of at least one or more of the *Yersinia* virulence factors, called Yops. The Yops, which are found in all three pathogenic *Yersinia* spp. and share homology to virulence factors in other bacterial pathogens, are secreted into host cells via a type III secretion machinery where they disrupt properties of mammalian cells. Most Yops, studied to date, have several phenotypes in cells in culture and/or multiple protein targets in biochemical assays. The long-term goals of this project are to understand the bacterial factors needed to establish an infection in specific tissues and the host defense mechanisms targeted by bacterial virulence factors. The specific goals of this proposal are to characterize which Yops are important in the GI tract and lymph tissues, which cell culture and/or biochemical phenotypes of the required Yops are needed for colonization in the GI tract and lymph, and to characterize the host defense factors in the GI tract that combat a *Yersinia* infection. This work has the potential to uncover heretofore-unknown aspects of host defense mechanisms in the GI tract. In addition, studies of yop mutant *Yersinia* strains in mouse strains with specific immune defects may indicate the facets of the immune system targeted by each Yop.

**Grant:** 1R21AI049438-01A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** HOSTETTER, MARGARET K PHD CLINICAL MEDICAL  
SCIENCES, OTHER  
**Title:** C3-Binding and -Degrading Proteins in *S. pneumoniae*  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2002/09/23-2004/09/22

DESCRIPTION (provided by applicant): *Streptococcus pneumoniae* remains a leading cause of morbidity and mortality in community acquired respiratory infections. The third component of complement, C3, stands as the central mediator of host defense in susceptible patients who lack anti-capsular antibody. Over the past 5 years, we have identified two C3-degrading enzymes from *S. pneumoniae*: CppA, which degrades the C3 beta-chain; and PhpA, which cleaves the C3 alpha-chain into previously unrecognized fragments. Neither proteinase has any homolog in the database, and both are expressed by a wide variety of encapsulated clinical isolates. Intranasal immunization of mice with recombinant rCppA reduced nasopharyngeal colonization with a serotype 3 organism. Immunization of mice with rPhpA significantly reduced bacteremia and increased survival; in separate experiments, immunization with rPhpA was more effective than the serotype 3 conjugate vaccine in reducing nasopharyngeal colonization. In addition to the protective effects of CppA and PhpA in vivo, cppA- and phpA- mutants are more susceptible to C3-mediated opsonophagocytosis in vitro than is the isogenic parent. This revised proposal focuses on the mechanisms by which CppA and PhpA enable *S. pneumoniae* to elude C3-mediated killing in blood and lung. In Specific Aim One, we will characterize the mechanism of proteolysis by which CppA degrades the C3 beta-chain using chromogenic substrates and standard protease inhibitors. Truncation constructs expressed in *Lactococcus lactis* will be used to map the active site. A cppA- mutant in an encapsulated serotype 4 will be constructed. Specific Aim Two will focus on PhpA, a 79 kDa proteinase that cleaves the C3 alpha-chain into novel fragments of 97 and 83 kDa. Possible biologic activities of these C3 fragments in inhibiting C3 or neutrophils will be assayed. Biochemical techniques will be employed to understand how full-length PhpA liberates an internal 20 kDa polypeptide that appears to account for the majority of C3-cleaving activity. A phpA- mutant in an encapsulated serotype 4 will be constructed. Specific Aim Three will use a standard killing assay and a double mutant to test for additive or synergistic effects of CppA and PhpA. Other opsonins in blood (fibronectin) and lung (surfactant protein A) will be assessed as potential substrates for CppA and PhpA. Specific Aim Four will employ cppA- and phpA- mutants in the encapsulated strain to understand whether the effects of CppA and PhpA on C3-mediated killing contribute to virulence in a rabbit model of pneumonia and bacteremia. This revised proposal will define the role of two potent immunogens in pneumococcal pathogenesis.

**Grant:** 1R21AI050785-01A1  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** GANTA, ROMAN R PHD  
**Title:** Cellular Immunity Against Ehrlichia chaffeensis  
**Institution:** KANSAS STATE UNIVERSITY MANHATTAN, KS  
**Project Period:** 2002/08/01-2004/07/31

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

**Grant:** 1R21AI051255-01  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** SUMMERSGILL, JAMES T PHD  
**Title:** Gene Expression of Persistent Chlamydia pneumoniae  
**Institution:** UNIVERSITY OF LOUISVILLE LOUISVILLE, KY  
**Project Period:** 2002/07/01-2004/06/30

DESCRIPTION (provided by applicant): Data have suggested a role for chronic Chlamydia pneumoniae infection in human atherosclerosis, however a direct causal role remains to be established. Elucidation of potential pathogenic mechanisms is critical to establishing whether C. pneumoniae actually possesses biological features consistent with a significant role in the initiation or exacerbation of this disease. Proteome analysis can be useful in elucidation of potential functions of expressed proteins, since changes in the bacterial proteome depend on growth stages, disease states or environmental conditions. Analysis of gene expression can also be determined via quantification of specific mRNA of a predetermined set of genes. A combination of transcriptomics and proteomics would provide a total picture of C. pneumoniae gene expression at any given time point. Evidence exists which localizes C. pneumoniae within cells of human atheromas. Thus, in order for C. pneumoniae to play a causative role in atherogenesis, it would need to persist within intimal tissue for extended periods of time, thereby stimulating a chronic inflammatory response. An alteration of the normal growth cycle can be induced, in vitro, leading to the induction of a persistent form of the organism. More importantly, an alteration in gene expression patterns, as well as the resulting Proteome, has been identified in the persistent forms of the bacterium. It is proposed that persistence of C. pneumoniae in cells of the developing atheroma, a microenvironment containing a multitude of host pro-inflammatory cytokines, could induce differential expression of specific bacterial proteins. Such proteins could serve as virulence factors or immunogens of C. pneumoniae. Modulated proteins of persistent C. pneumoniae in cell culture will be identified by mRNA and proteomics analysis, sequenced, expressed in an appropriate vector and specific antibodies. Such reagents could be used to probe infected cell cultures as confirmation step prior to their future use in probing human tissue specimens suspected of harboring persistent C. pneumoniae.

**Grant:** 1R21AI051561-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** KERNODLE, DOUGLAS S  
**Title:** Pro-Apoptotic Tuberculosis Vaccine  
**Institution:** VANDERBILT UNIVERSITY NASHVILLE, TN  
**Project Period:** 2002/06/15-2003/05/31

**DESCRIPTION:** (Provided by Applicant) A major hurdle in the development of effective vaccines against pathogens that reside within macrophages, including *Mycobacterium tuberculosis*, is how to deliver exogenous antigens in a manner that stimulates a protective cellular immune response. Recent investigations involving antisense mutants of *M. tuberculosis* H37Rv that have diminished production of superoxide dismutase (SOD) and exhibit promising activity as a vaccine prototype suggest that the mechanism of vaccine efficacy may be apoptosis-associated cross-presentation of microbial antigens to CD8<sup>+</sup> lymphocytes via MHC Class I pathways. The goals of the current proposal are first, to characterize the cellular and cytokine responses in the lung observed early after infection with SOD-diminished *M. tuberculosis*, as rapid pulmonary interstitial infiltration with mononuclear cells undergoing apoptosis appears to be a process unique to the SOD-diminished strains that is not observed during infection with either virulent *M. tuberculosis* or the current vaccine strain for tuberculosis, BCG. This should define the conditions under which antigen cross-presentation occurs in vivo, yielding information that may be useful for a variety of vaccines. The second goal is to construct non-reverting SOD-diminished mutants of H37Rv and BCG by replacing the wild-type SOD allele with mutant alleles, some of which encode enzymatically less efficient mutants of SOD. This should yield a SOD-diminished vaccine candidate that is stable and safe enough for administration to man. The third goal is to determine the optimal level of SOD production for maximum vaccine efficacy and the immune correlates of protection. Diminishing the production of factors produced by intracellular pathogens that inhibit macrophage apoptosis is a strategy for making new vaccines that achieve MHC Class I antigen presentation. This should have implications not only for tuberculosis but for other infectious diseases in which CD8<sup>+</sup> T-cell responses are a critical component of a protective immune response.

**Grant:** 1R21AI051696-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** BRIKEN, VOLKER PHD  
**Title:** Genetic Screens for Virulence Factors of Mycobacteria  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2002/06/01-2004/05/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis (M.tb) infects one-third of the world's population and claims the lives of two to three million people each year. Its success is achieved by its ability to persist in the hostile intracellular environment of infected macrophages. After invasion, M.tb manipulates the phagocytic pathway of the host cell to inhibit the maturation of the phagosome into a phagolysosome. In addition, M.tb inhibits the apoptotic response of infected macrophages. These effects are likely to represent a highly evolved strategy that is used by M.tb to evade the host immune response. Although some cellular proteins have been characterized as targets of M.tb, it is currently unclear which bacterial proteins or lipids mediate the interactions. The proposed research project aims at filling this gap in our knowledge by focusing on the identification of proteins or lipids of M.tb that are implicated in either inhibition of maturation of the bacterial phagosome or inhibition of the apoptotic response of the host cell. The recent advances in the genetic manipulations of mycobacteria will be used to randomly mutagenize green fluorescence protein (GFP)-labeled M.tb and screen for mutants deficient in inhibiting phagosome maturation using a newly developed FACS-based, high-throughput assay. In a second approach, Mycobacterium smegmatis, a nonpathogenic mycobacterium deficient in inhibiting phagosome maturation and apoptosis of the host cell, will be complemented with M.tb genes and clones that have gained this capacity will be selected. Identification of M.tb genes essential for persistence of the pathogen will provide important targets for the development of new drugs for the treatment of tuberculosis and the development of new attenuated strains of M.tb that may be used as vaccines.

**Grant:** 1R21AI051707-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** HUSSON, ROBERT N MD  
**Title:** Signal Transduction and Latency in M. tuberculosis  
**Institution:** CHILDREN'S HOSPITAL (BOSTON) BOSTON, MA  
**Project Period:** 2002/06/01-2004/05/31

DESCRIPTION (provided by applicant): Latency in the human host is a unique aspect of the pathogenesis of M. tuberculosis infection with important implications for prevention, treatment and elimination strategies. The understanding of latency is limited, though recent insights into the adaptation of M. tuberculosis to the environment of the macrophage, and to oxygen limitation may have important implications for latency in the human host. The hypothesis of this research is that gene expression that is regulated in concert with cell division is likely to play an important role in the transition of bacterial physiology to the latent state, and in the emergence of the organism from the latent state. The research proposed in this R21 application is designed to begin to characterize the role of two serine/threonine kinases, PknA and PknB, in the regulation of M. tuberculosis gene expression. These kinases have the structure of signal transduction kinases with predicted extracellular receptor domains and intracellular kinase domains. Their location near the bacterial origin of replication in an operon containing genes involved in cell wall biosynthesis suggests that their expression is linked to cell division. To begin to investigate the function of these regulatory genes in the context of active replication and latency, and their potential as targets for therapeutic intervention, two specific aims are proposed: 1) To undertake an initial functional characterization of pknA and pknB, including the effects of these regulatory proteins on growth, viability and patterns of gene expression and 2) To identify protein targets of the kinase domains of the serine/threonine kinases PknA and PknB. Approaches used in this research include regulated expression of pknA and pknB wild type and kinase-inactive (dominant negative) constructs, expression library cloning, and two-dimensional protein gel electrophoresis. The goal of this research is to gain initial insight into the function of these genes to provide a framework for future long-term investigation of their role in M. tuberculosis infection and latency.

**Grant:** 1R21AI051727-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** CHAN, JOHN R MD  
**Title:** M tuberculosis truncated hemoglobins in persistence  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2002/08/01-2004/07/31

DESCRIPTION (provided by applicant): Recrudescence of latent tuberculous infection contributes significantly to the pathogenesis of disease caused by *Mycobacterium tuberculosis* (Mtb). The mechanisms by which the tubercle bacillus establishes latency and later reactivates are, however, poorly understood. Two truncated hemoglobins (trHb's), HbN and HbO, encoded by the gene *glbN* and *glbO*, respectively, exist in Mtb. Initial characterization of HbN and HbO in *M. bovis* BCG has shown that: i) *glbN* and *glbO* are differentially expressed --the expression of HbN in vitro is most prominent in the stationary phase of growth, while HbO is invariably detected throughout the various growth phases; ii) both trHb's have high affinity for oxygen, albeit via different mechanisms; iii) HbN and HbO can detoxify nitric oxide (NO) by conversion of the nitrogen oxide to nitrate; significantly, a BCG deletion mutant of *glbN* is markedly attenuated for its ability to consume NO; iv) the function of HbO may be essential. Based on these findings, we propose to test the hypothesis that HbN and HbO are required for the survival and/or persistence of Mtb within the host. This survival/persistence-promoting attribute can be due to the ability of HbN and HbO to detoxify the antimycobacterial NO. In addition, by virtue of their ability to bind oxygen with high affinity, HbN and HbO can function as an oxygen reservoir in the relatively anaerobic environment of the tuberculous granuloma, thereby optimizing the functions of critical intracellular oxygen-dependent enzymes. Finally, the ability of these trHb's to avidly bind oxygen may protect Mtb against oxidative damage. To begin testing these hypotheses, we will take a genetic approach, by generating *glbN* and *glbO* mutants, to rigorously test the in vivo significance of Mtb trHb's in survival and/or persistence using murine experimental TB models. We will also evaluate the roles of HbN and HbO in Mtb respiration and in protection against the adverse effects of NO on the respiratory process. Establishment of the significance of HbN and HbO in persistence and unraveling the biochemical and physiochemical properties of these hemeproteins will set the stage for developing novels anti-tuberculous agents effective against Mtb, particularly those in the dormant state.



**Grant:** 1R21AI052141-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** NEELY, MELODY N BS  
**Title:** Streptococcal-Zebrafish Model of Bacterial Pathogenesis  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 2002/09/15-2003/09/14

DESCRIPTION (provided by applicant): Streptococcal pathogens continue to evade concerted efforts to decipher clear-cut virulence mechanisms, although numerous genes have been implicated in pathogenesis. A single species can infect a diversity of tissues, suggesting the expression of specific virulence factors based on the local tissue environment or stage of infection. Our long-range goal is to identify the interactions that occur between the host and pathogen that lead to activation of virulence mechanisms and contribute to specific streptococcal disease states. The objective of this application is to characterize specific virulence mechanisms utilized within various tissues in vivo by employing a unique animal model, the zebrafish (*Danio rerio*). We will accomplish this by studying infection by two streptococcal species that represent two forms of streptococcal disease: a natural pathogen of both fish and humans, *Streptococcus iniae* and a human-specific pathogen, *Streptococcus pyogenes*. While *S. iniae* primarily causes systemic disease in the zebrafish following intra-muscular injection, *S. pyogenes* causes a locally spreading necrotic disease confined to the muscle. By studying pathogens that are virulent for both fish and humans and that mediate disease states in the zebrafish that are identical to those found in human streptococcal infections, we will be able to identify common virulence strategies shared by a number of Gram positive pathogens. The central hypothesis is that streptococcal pathogens respond to their host by initiating specific virulence mechanisms based on the local tissue environment or host-specific factor expressed within that tissue. We propose to: (1) identify and characterize bacterial proteins that interact with the host in vivo to cause specific disease states; (2) characterize the role in pathogenesis of proteins previously implicated in virulence; and (3) identify the factors required for initial colonization by streptococcal pathogens.

**Grant:** 1R21AI052316-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** KISICH, KEVIN O PHD  
**Title:** Antimicrobial Peptides in Resistance to Tuberculosis  
**Institution:** NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Infections with drug resistant strains of *Mycobacterium tuberculosis* and most strains of *Mycobacterium avium* are challenging to treat chemotherapeutically. As HIV infection and the AIDS epidemic spreads, adequate treatment of mycobacterial infections in both immunocompetent and immunocompromised patients becomes increasingly difficult. Additional understanding of innate and immune mechanisms of resistance as well as new antibiotics will be important for designing new, more effective treatment regimens for mycobacterial infection. We hypothesize that small, cationic human antimicrobial peptides (AMP), defensins and cathelicidin stored in granules of human polymorphonuclear leukocytes (PMN), and secreted from epithelia play important roles in resistance to mycobacterial infection, and may be adaptable for chemotherapeutic use. Human neutrophil peptides 1,2, and 3 (HNP 1,2, and 3) have shown to be anti-mycobacterial for laboratory and clinical isolates of *M. tuberculosis* and *M. avium* in vitro, and we have confirmed this in our laboratory. We propose to extend these observations by fully characterizing the anti-mycobacterial activity of human AMP, examining the relationship between binding of AMP to mycobacteria and anti-mycobacterial activity, and evaluating the relationship between resistance to human AMP and pathogenicity. We will also examine pharmacological interactions between human AMP and first-line anti-tuberculosis drugs for mycobacteria in liquid and macrophage cultures. The proposed studies are designed to fill an important gap in our knowledge of how mycobacteria evade the innate immune system. Understanding of the relative sensitivities of mycobacteria will help us understand how mycobacteria survive their first encounter with the antimicrobial peptides in pulmonary secretions and in responding neutrophils. Correlation of AMP sensitivity with binding of the peptides will help determine whether and how AMP sensitivity is related to pathogenicity. In addition, the proposed studies will define the potential of AMP to participate in the anti-mycobacterial response of human macrophages, or whether intracellular mycobacteria are protected from exposure to AMP. These studies will further our understanding of how endogenous anti-mycobacterial substances may enhance resistance to infection, and will also reveal whether these substances may be adaptable to chemotherapeutic use for treatment of mycobacterial infections.

**Grant:** 1R21AI052356-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** KING, C H PHD  
**Title:** Cellular Necrosis Induced by Mycobacterium tuberculosis  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 2002/09/15-2003/12/31

DESCRIPTION (provided by applicant): A key step in the pathogenesis of M tuberculosis is its ability to cause caseating necrosis, parenchymal lung destruction, and cavity formation, which develop into the characteristic necrotizing bronchointerstitial pneumonia and bronchiolitis of tuberculosis. M tuberculosis is cytotoxic to epithelial cells in vitro, and we have shown that this cytotoxicity is associated with cell membrane permeation to lactose dehydrogenase and is mediated by necrosis of lung epithelial cells after infection with virulent mycobacteria (Dobos, K. M., Quinn, F. D. and King, C. H. 2000, Infect. Immun. 68:6300-6310). Interestingly, the attenuated M bovis BCG does not induce necrosis in this epithelial cell model suggesting that necrosis is related to the virulence of mycobacteria. Our working hypothesis is that M tuberculosis possesses factors that cause necrosis. We intend to identify the genes that encode or synthesize such factors and determine their functions. Towards this goal, we have been successful in isolating two such (necrosis-deficient) mutants with insertions into genes that have no known function by screening a transposon library of the Erdman strain of M tuberculosis (TN5370) for mutants that have lost their ability to cause cell membrane permeation and necrosis. Both nec mutants possess extremely interesting phenotypes when grown in mice. The first mutant (necA) appears to be highly attenuated for growth and virulence in SCID mice. This is an important result as it suggests that we have identified a gene whose product either directly causes necrosis or induces necrosis and thus should enhance our understanding of tuberculosis pathogenesis. Interestingly, the second mutant (necB) appears to kill SCID mice more rapidly than the parental strain. We intend to characterize these mutants, characterize the functions of the gene products, and extend this mutant isolation strategy to identify a large battery of mutants defective for necrosis of host cells.

**Grant:** 1R21AI052374-01  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** CAMILLI, ANDREW BS  
**Title:** Study of S pneumoniae Virulence Gene Regulation  
**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA  
**Project Period:** 2002/09/15-2004/09/14

DESCRIPTION (provided by applicant): The overall goal of our research is to understand the pathogenicity of *Streptococcus pneumoniae*, the most common cause of bacteremia, bacterial meningitis, otitis media and community-acquired pneumonia in the U.S.A. Current capsule-based vaccines, which only contain a subset of the capsular types in circulation, do not provide adequate protection from pneumonia and otitis media, which account for the majority of *S. pneumoniae* morbidity. Despite more than a century of research, understanding of *S. pneumoniae* virulence factors is limited. Furthermore, almost nothing is known concerning the regulation of *S. pneumoniae* virulence factors during infection. These limitations, plus an increasing incidence of antibiotic-resistance mandate increased study of the pathogenicity of this organism. We have completed a large-scale screen that resulted in the identification of 233 genes that are essential in a murine model of pneumonia. Additionally, we tested the importance of each of these genes in murine models of bacteremia and nasopharyngeal carriage. Included among these novel virulence factors are 21 surface proteins, and 20 putative regulators that we hypothesize coordinate tissue-specific virulence gene expression. The first goal of the proposed work is to gain an understanding of both the regulation and mechanisms of action of two factors hypothesized to localize to the bacterial surface and interact with host components. Mutational analyses and virulence assays will be combined to define their interacting domains. The second goal is to identify major virulence gene regulons and their modes of coordination during infection. Five putative transcription factors identified in our screen will be placed under inducible expression, and the subset of genes regulated by each will be determined by transcriptional profiling on microarrays. For selected genes, the level of expression during nasopharyngeal carriage in mice and humans will be determined using quantitative RT-PCR. The requirement for each cognate regulator for expression of these virulence genes during infection of mice will be confirmed. These studies will enhance our knowledge and understanding of *S. pneumoniae*-host interactions and virulence mechanisms, and will constitute the first broad study of *S. pneumoniae* virulence gene regulation. These studies will aid in the development of novel vaccines, and will suggest new targets for antimicrobial drug development.

**Grant:** 1R21AI052458-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** SCHLESINGER, LARRY S MD  
**Title:** Altered M. tuberculosis Mannosylation and the Macrophage  
**Institution:** UNIVERSITY OF IOWA IOWA CITY, IA  
**Project Period:** 2002/09/15-2002/10/31

DESCRIPTION (provided by applicant): Tuberculosis continues to cause tremendous morbidity and mortality throughout the world's population. Critical in establishment of a M. tuberculosis (M.tb) infection within its human host are entry and survival in the macrophage. The macrophage mannose receptor (MR) participates in the phagocytosis of virulent strains of M.tb. Components of the M.tb cell wall serve as ligands for host cell receptors and can modulate host microbicidal and inflammatory responses. The M.tb cell envelope is heavily mannosylated containing lipoglycans such as lipoarabinomannan (LAM) which serves as a ligand for the MR. We hypothesize that the nature of surface mannosylation of M.tb has a major impact on the ability of M.tb to interact with the MR as well as to modulate macrophage function and consequently host responses, enabling the establishment of infection. PimB was recently described as M.tb phosphatidyl myo-inositol monomannoside transferase (pimB). We have used allelic-exchange to inactivate pimB in M.tb strain Erdman. Macrophages display marked cellular adhesion following infection with wild-type M.tb. In contrast, macrophages infected with the pimB mutant display minimal cellular adhesion and a significant increase in the rate of macrophage death. We have developed an assay in which Salmonella mannose-specific binding pili agglutinate M.tb LAM coated microspheres that we will develop as a screen for alterations in M.tb surface mannosylation. We propose to further characterize the role of pimB and other selected enzymes potentially involved in mannosylation of M.tb surface molecules in the biology of the M.tb-host interaction, to develop a novel screening strategy for M.tb clones altered in surface mannosylation, and to evaluate these bacterial clones for anomalous host cell interaction. Our specific aims are to: 1A. Determine the mechanism for reduced homotypic adhesion and increased rate of macrophage death following infection with the pimB mutant of M.tb: 1 B. Determine the biochemical nature of the pimB mutation. Analyze the structure of LAM and other mannosylated cell wall glycoconjugates from wild type, the pimB mutant, and pimB overproducing M.tb strains. 2. Perform transcription and genetic studies of genes encoding the biosynthetic enzymes of LAM and mannose glycoconjugates: 2A. Quantify the level of transcription of pimB and its homologues in M.tb grown in broth, solid medium and within human macrophages using the ABI 7700 (TaqMan) "real-time" quantitative PCR system; 2B. Construct and analyze genetically defined M.tb strains with alterations in the mannose biosynthetic genes. 3). Utilize Salmonella mannose-binding (type 1) pili to screen for M.tb mutants and clones respectively altered in surface mannosylation from an M.tb transposon library and M. smegmatis library complemented with M.tb genes to characterize the effect of alterations in bacterial mannosylation on macrophage interaction. The assembled investigators will combine techniques in genetics, biochemistry, and cell biology to accomplish the goals of this proposal.

**Grant:** 1R21AI052777-01  
**Program Director:** LAUGHON, BARBARA E.  
**Principal Investigator:** WELCH, JOHN T PHD  
**Title:** A New Target for Anti-tuberculosis Agents, FAS 1  
**Institution:** STATE UNIVERSITY OF NEW YORK AT ALBANY, NY  
ALBANY  
**Project Period:** 2002/08/01-2004/07/31

DESCRIPTION (provided by applicant): As a sentinel disease for the diagnosis of AIDS, early reports of tuberculosis complicating AIDS seemed to indicate the disease was a result of reactivation of a previous infection, however today the picture of tuberculosis as a complication of AIDS has changed dramatically. The development of multidrug resistant strains of *M. tuberculosis* and the prospect of nosocomial transmission make the development of new, better tolerated therapies ever more important. It is the inclusion of pyrazinamide (PZA), an agent with a unique sterilizing activity, with isoniazid and rifampin (RIF) in current treatment regimens that constitutes the basis for 6-month short course therapy for *M. tuberculosis* (Mtb). Surprisingly for a clinical agent, a detailed understanding of the mechanism of action of PZA continues to elude researchers. Recently the surprising observation was made that an analog of PZA, 5-chloropyrazinamide (5-CI-PZA), inhibits fatty acid synthetase I, (FAS 1) in *Mycobacterium tuberculosis*. This observation has been confirmed independently in other laboratories. In prokaryotes, the synthetase is typically composed of at least seven peptides that represent the individual enzyme components and are generally classified as Type II synthases. However in mammals and mycobacteria, the synthase activity is carried out by single high-molecular weight, multifunctional peptide chains which are known as Type I synthases. In cell free extracts of *Saccharomyces cerevisiae* not only was the activity of 5-CI PZA confirmed to be very comparable with that of the well-known inhibitor cerulenin in blocking the activity of FAS 1 it was also found that other pyrazinamide analogs also apparently inhibit FAS 1. The hypotheses to be tested in this proposal are that characterization of the mechanism of 5-CI-PZA on the inhibition of FAS 1 can lead to the development of better anti-mycobacterial agents and, secondly, that this inhibition process will be sensitive to structural variations of the pyrazinoic or nicotinic acid structure. The utility of the observed anti FAS activity can be enhanced by developing an understanding of the nature of the FAS 1 inhibition process so that this activity can be employed in the design of novel anti-tuberculous agents. As it is known that treatment of microbes with type I FAS inhibitors increases the sensitivity of those microbes to downstream inhibitors in a synergetic fashion, the development of anti-mycobacterial agents that act on FAS 1 and that can be used in combination with the aforementioned downstream agents may have a profound effect on improving tuberculosis treatment.

**Grant:** 1R21AI052821-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** LOUIE, LESLIE G PHD  
**Title:** Genetics of TB: Cytokine Response and Iron  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA  
OAKLAND  
**Project Period:** 2002/04/01-2003/07/31

DESCRIPTION (provided by applicant) Evidence from animal models and from other Mycobacterial diseases in humans (e.g., leprosy) implicates a role for cytokine profiles in determining the outcome of human infection with Mycobacterium tuberculosis (M.tb.). These profiles are influenced in part by the host's ability to respond to infection, antigenic stimulation of the host, and underlying predisposing conditions. HIV infection is a major predisposing factor in TB and is epidemic in Zimbabwe. Iron overload has also been documented in Zimbabwe and evidence exists that iron plays a role in host response to M.tb. infection. Thus, the immune response to M.tb. infection is complex and is likely to be influenced by both genetic and environmental factors. Our ongoing study, "Genetic Contribution of Host and Pathogen in African TB," (AI 40019) examines variation at host immune response genes in M.tb. infected controls and TB cases, both with and without HIV co-infection. The current application will expand the ongoing study to allow dietary nutrients and cytokine responses to be tested, and it will permit a greater range of immune response genes to be examined. The sample of extrapulmonary TB (EPTB) cases will be augmented in an effort to understand the role of host genetic risk factors and cytokine responses in disease progression among infected individuals. We will address the following hypotheses: 1) Genetic variants that affect the expression or function of cytokines and iron metabolism will influence risk of TB; 2) Impaired IFN- and IL-12 responses occur in tuberculosis and are more profound when extrapulmonary or disseminated disease exists; 3) HIV-1 stage of infection can influence the type of cytokine response to M. tb. infection; and 4 ) Differences in iron status and iron metabolism influence risk of TB. To this end, we will recruit HIV+ and HIV- patients with PTB, HIV+ patients with EPTB (pleural effusion or lymphadenitis), or HIV+ and HIV- controls without TB who are PPD skin-test positive (n=125 per group, total n=750 subjects). These subjects will be screened for HLA class I and II genes, cytokine genes, and iron metabolism gene variation. Cytokine production in response to stimulation by mycobacterial antigens and markers affecting iron will be measured. HIV-1 infection will be staged by viral load and CD4 cell count. By understanding the role of host genetic variation, cytokine response, HIV infection, and iron in susceptibility to TB infection and disease progression, public health control measures can be pursued, including vaccine design, vaccine testing, cytokine therapy, and dietary recommendations.

**Grant:** 1R21AI052847-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** PAULI, GUIDO F PHD  
**Title:** Metabolome of non-replicating M. tuberculosis  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): It is now generally accepted that new drugs are needed to make global tuberculosis control a reality. The completion of the sequence of the M. tuberculosis genome has laid the foundation for greatly accelerating new drug discovery. While a limited number of studies of the TB transcriptome and proteome have been reported, these global macromolecular analyses are still not able to contribute directly to drug discovery except where gene/protein function are known or protein crystal structures are available. Elucidating the full complement of low molecular weight compounds in an organism -the metabolome - is becoming recognized as a complementary and perhaps the most tractable approach to a comprehensive understanding of any pathogen including the insight necessary for rational drug discovery. This R-21 proposal seeks to define the metabolome of the tubercle bacillus in the stage of non-replicating persistence (NRP), the physiological state considered to be responsible for the required long treatment duration for TB. The metabolome will be elucidated by high-resolution chromatography (CCC, LC) and -spectroscopy (MS, NMR). Initial studies will utilize a high biomass of BCG to evaluate several separation schemes in optimizing the resolution of (secondary) metabolites. This protocol will then be used to define the metabolome of M. tuberculosis in NRP. Subsequent experiments will focus on understanding the growth-phase specificity of unique metabolites produced under NRP. The information obtained will not only complement the extensive knowledge of the chemistry of the cell wall but is also expected to 1) identify novel secondary metabolites possibly related to those recently described for other mycobacteria, 2) help to clarify events and identify low MW markers during the metabolic shift to a low oxygen environment especially considering that >70% of genes up-regulated during this adaptation are of unknown function, 3) provide leads for drug development through analoging around TB-specific compounds not known to occur in mammalian cells and 4) assist in the understanding of the mechanism of action of newly discovered anti-TB agents. The operating hypothesis for this study is that elucidating the metabolome of Mycobacterium tuberculosis will overcome limitations inherent in global macromolecular analyses with respect to gaining key insights into dormancy and drug discovery.



**Grant:** 1R21AI053114-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** MUSTELIN, TOMAS MD  
**Title:** Molecular mechanism of immune evasion by Yersinia pestis  
**Institution:** BURNHAM INSTITUTE LA JOLLA, CA  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Yersinia pestis, the etiologic agent of plague or Black Death, has in historical times caused devastating pandemics unrivaled by any other infectious disease. Due to its very rapid replication and effective immune evading capacity, Yersinia pestis has recently been recognized as a potential tool for bioterrorism. In particular, an aerosolized delivery of the bacterium could cause a rapid and fulminant pneumonic infection, which subsequently may spread from person to person. Although several vaccines exist, and Yersinia usually is sensitive to streptomycin and tetracycline type antibiotics, the pneumonic form of the disease is difficult to treat and still often results in death. Additional supportive treatments may significantly reduce this lethality, especially in the case of massive exposure of a population to weaponized Yersinia. This grant application focuses on a key component of the molecular machinery by which Yersinia pestis evades the immune system, a highly active protein tyrosine phosphatase (PTPase) termed YopH. In infected hosts, the bacteria multiply in lymph nodes, where they adhere to T and B cells and inject them with YopH. Inside the lymphocytes, YopH efficiently inhibits lymphocyte activation and the development of an immune response. It has been suggested that YopH interferes with early T cell antigen receptor signaling, but the precise mechanisms remain unknown. This application will address the following two Specific Aims. Specific Aim 1 is titled "Molecular targets of YopH in T cell antigen receptor signal transduction". These studies will utilize our familiarity with early T cell antigen receptor-induced signaling events and the molecular mechanisms by which different PTPases regulate these events. We plan to determine the molecular mechanism by which YopH inhibits TCR-induced T cell activation using substrate-trapping technology, proteomics, tryptic peptide mapping, confocal microscopy and functional assays and read-outs for T cell activation. Specific Aim 2 is titled "Development of a YopH-specific inhibitor". In this Specific Aim, we will develop a YopH-specific small molecule inhibitor by first screening the Burnham Institute's chemical library using a colorimetric assay and the BioMek2000 robotic station that was established for this purpose. After two levels of screening and counter-screening with several other PTPases, we anticipate being left with a few inhibitory compounds, which will be evaluated for efficacy in the T cell signal transduction assays established in Specific Aim 1. The results obtained in this study will enable us to better understand the molecular mechanisms by which Yersinia pestis evades the adaptive immune system using the YopH PTPase. This information, together with a specific YopH inhibitor that works in the same T cell activation model, will represent valuable progress towards the goal of designing a drug to combat plague mortality.

**Grant:** 1R21AI053269-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** JANDA, KIM D  
**Title:** Biocombinatorial Strategies Against Botulinum Toxin  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): The current warfare and terrorist activities around the world, in particular directed against the American people and infrastructure, necessitates a vastly enhanced defense against potential weapons of mass destruction. Among the most lethal and readily deployed means that must be considered are the various toxins of biological origin. In this regard, an often cited scenario is the use of botulinum toxin (BoTox), the causative agent of the clinical condition of botulism, which can have a high fatality rate by either oral consumption or inhalation. New and improved methods of rapidly and conveniently detecting small amounts of BoTox in its various forms are needed, as well as therapeutics for protection against its deadly effects. We propose "biocombinatorial strategies" developed in our laboratory, which refer to human antibodies and cyclic peptides derived from novel phage-display libraries using various selection techniques, against the threat of BoTox. Each of these biotechnological reagents could serve for diagnostic and medicinal purposes, in other words, for detection and/or protection. Specifically, we will use our proprietary antibody and peptide libraries for (1) selection of antibodies and cyclic peptides against BoTox by routine panning, (2) selection of antibodies and cyclic peptides against BoTox by BIAcore panning, (3) characterization of selected antibodies and cyclic peptides by ELISA, (4) characterization of selected antibodies and cyclic peptides using BIAcore, (5) detection of trace amounts of BoTox using antibodies/peptides and BIAcore, and (6) evaluation of selected antibodies and cyclic peptides using the PC12 cell line. The research described provides a foundation for highly sensitive detection and monitoring of BoTox in progenitor and toxic forms and for passive immunotherapy against the active toxin.

**Grant:** 1R21AI053285-01

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

**Principal Investigator:** RAVETCH, JEFFREY V MD INTERNAL  
MED:INTERNAL MEDICINE  
UNSPEC

**Title:** Novel Strategies for Y pestis Immunotherapy

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

**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): A better understanding of the pathophysiology of Y. pestis and its interaction with the host immune system is central to the development of novel approaches for the prevention and treatment of bioterrorism associated plague. While much work has been done on the interrelationship between the bacterium and the innate immune system of the host, little is known about the mechanisms by which the bacterium is able to evade adaptive immune responses. The focus of this application will be to identify the specific effectors (Yops) used by Y. pestis to prevent adaptive immunity from developing during an infection. New experimental systems will be developed which will permit the dissection in vitro and in vivo of the effects of Yops on the adaptive immune response. The interactions of the phosphotyrosine phosphatase, YopH, with the host adaptive response will be analyzed as a prototype of this approach. Four aims will be pursued in this program. In Specific Aim 1, B cell, T cell and macrophage lines expressing Yop H will be generated and analyzed for their ability to mediate antigen dependent and independent activation and effector responses. Specific Aim 2 will further define the specific signaling pathways perturbed by Yop H expression in B cells by using DT40 clones deficient in specific signaling components that also express Yop H. These in vitro studies will provide the rationale for generating transgenic mice in Specific Aim 3 in which the expression of Yop H is targeted to specific lymphoid and myeloid compartments. These animals will be evaluated in Specific Aim 4 for their ability to develop a mature immune system and for their ability to respond to thymic dependent and independent antigens, through appropriate antigen presentation, T cell activation and B cell stimulation, affinity maturation and expansion. This experimental approach will then permit, in the future, for similar studies on other Yops and for the infection of specific Yop expressing transgenics with Y. pestis mutants defective in virulence. This approach will thus permit the evaluation of the interaction of the pathogen with adaptive immune pathways by bypassing the effects on innate responses.

**Grant:** 1R21AI053287-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** BLANKE, STEVEN R PHD BIOCHEMISTRY  
**Title:** Blocking Cellular Intoxication by Bacterial Toxins  
**Institution:** UNIVERSITY OF HOUSTON HOUSTON, TX  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): BoNTs are a dangerous bioterrorism threat due to their extreme potency and lethality, as well as their ease of production and transport. If untreated, poisoning by the BoNTs can progress to flaccid paralysis and death due to respiratory failure. However, timely post-exposure intervention can limit the effects of the circulating toxin. The overall, long-term research objective is to generate a novel class of therapeutics that can be administered to individuals who have been poisoned by BoNT. The strategy, as described in this application, will be to develop dominant-negative mutants of one form of the toxin, BoNT/A, which will interact with and inactivate complexes of wild type toxin. BoNT/A is composed of two defined fragments. The H chain facilitates the transport of a second toxin fragment, the L chain, into target cells. The strategy is based on the hypothesis that transport of the BoNT/A L chain, which is an essential step in the cellular intoxication mechanism, is mediated by pH-triggered unfolding and membrane insertion of toxin oligomeric complexes. Experimental and computational approaches will be used to develop a model for the mechanism of H chain-mediated membrane transport of the L chain. This model will be tested by altering the amino acid sequence of the H chain in a manner predicted to interfere with membrane transport. Specifically, intramolecular disulfide linkages will be engineered within the H chain to limit movement of the polypeptide backbone as a result of pH triggered conformational changes. Moreover, positively charged amino acids will be introduced throughout the H chain, which would be predicted to be unfavorable for membrane insertion of the BoNT/A H chain. Wild type and mutant forms of the H chain will be expressed as recombinant proteins, and each mutant will be tested for dominant-negative inhibitory activity in the presence of wild type toxin. Simultaneously, structure-function relationships important for translocation will be identified as an important prerequisite for future design of dominant-negative based inhibitors. A milestone of this work will be the identification of one or more dominant-negative BoNT/A mutants that will block action of wild type toxin using in vitro model systems. The results from this research will establish the groundwork and justification for future development and in vivo testing of these novel inhibitors using established animal models.

**Grant:** 1R21AI053292-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** PIZZO, SALVATORE V  
**Title:** Alpha2-Macroglobulin-PA Complexes: Novel Anthrax Vaccin\*  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): An urgent need exists for the identification and development of novel approaches for delivering protective antigens as more effective vaccines against a variety of agents which might be used as biological weapons, including anthrax, botulism and plague. The long-term objective of the proposed application is to develop a new generation of vaccines against biological agents based on a totally natural, non-reactogenic adjuvant, alpha2-Macroglobulin (alpha2M). Alpha2M has been shown to greatly enhance immunogenicity of a number of antigens. The development of alpha2M adjuvanted vaccines will significantly impact healthcare in both the U.S. and the world, as it will allow a new generation of vaccines, both prophylactic and therapeutic, based on protein subunits, which can be produced inexpensively and are intrinsically safer to use. PA, recognized as the major protective antigen in the current anthrax vaccine (AVA, Anthrax Vaccine Absorbed), will be used as a prototypical subunit candidate for the proposed studies. The specific aims of the proposed studies are to (1) identify the optimal size of Bacillus anthracis PA and the optimal conditions for its covalent incorporation into rabbit alpha2M; (2) determine the ability of various complexes of PA covalently coupled with a2M to generate neutralizing antibodies to anthrax toxin; and (3) determine if the immunogenicity of PA complexed with alpha2M can be enhanced by combination with existing adjuvants. Specifically, full-length PA (83 kDa, rPA83) will be expressed in E. coli and purified to homogeneity. Proteolysis of rPA83 will be used to generate "nicked PA" (63 kDa, rPA63), or a carboxy-terminal fragment containing the receptor-binding domain (47 kDa, rPA47). a2M will be purified to homogeneity from rabbit plasma. Studies will determine the efficiency of incorporation of rPA of varying size into rabbit a2M under various conditions. Complexes of rabbit a2M and rPA (alpha2M-rPA) will be then be used to immunize rabbits and will be compared for immunogenicity against rPA alone absorbed onto alum. Sera from immunized rabbits will be evaluated for anti-rPA titers (based on ELISA), immunoglobulin isotypes, and ability to block anthrax toxin mediated macrophage cytotoxicity (neutralizing activity). Alpha2M-rPA complexes, which generate neutralizing titers, will be further evaluated in combination with other adjuvants. These studies will provide a new anthrax vaccine candidate with both improved immunogenicity and decreased reactogenicity.

**Grant:** 1R21AI053298-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** MILLER, VIRGINIA L  
**Title:** RovA regulon of *Yersinia pestis*  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2002/09/27-2004/08/31

DESCRIPTION (provided by applicant): *Yersinia pestis* is a Gram-negative bacterial human pathogen that is the causative agent of plague. Historically *Y. pestis* has been responsible for significant human morbidity and mortality. Due to the high mortality rates and severe disease caused by *Y. pestis*, it has emerged as an agent of biological warfare and bioterrorism. A more detailed understanding of the pathogenesis of *Y. pestis* will facilitate the identification of targets for vaccine development and treatment. Currently, aside from studies of the type III secretion system and effectors encoded on the virulence plasmid, very little is known about the pathogenesis of *Y. pestis* at the molecular level. Much of what is known about the pathogenesis of *Yersinia* comes from the investigation of the other two human pathogens in the genus, *Y. enterocolitica* and *Y. pseudotuberculosis*, both of which are primarily enteric pathogens but are capable of causing systemic infection much like *Y. pestis*. The enteropathogenic *Yersinia* are some of the best-characterized pathogens due to the ease of genetic manipulation and the use of the mouse as a model. Murine yersiniosis recapitulates most aspects of disease in humans allowing for a detailed analysis of the pathogenesis. All three pathogenic species of *Yersinia* have virulence plasmids that are required for full virulence. However, using *Y. enterocolitica* we have employed a wide variety of techniques to identify numerous virulence factors that are encoded on the chromosome. One of these genes is a transcriptional regulator of the MarR family called *rovA*. *RovA* is present in all pathogenic species of *Yersinia* and has been demonstrated to regulate numerous virulence factors in *Y. enterocolitica*. The *Salmonella* homologue of *RovA* also regulates several virulence factors in *Salmonella*. Although *RovA* and *SlyA* are clearly regulators of virulence, the virulence factors regulated are not the same, suggesting that *RovA* regulated genes may differ depending on the species. Together these observations lead us to hypothesize that the *Y. pestis* *RovA* is a regulator of virulence and that by studying the role of *RovA* in the virulence of *Y. pestis* we will gain a greater understanding of the pathogenesis of plague. Specifically we propose: (1) Analysis of the role of *RovA* in the virulence of *Y. pestis*, and (2) Identification of the *Y. pestis* *RovA* regulated gene products; we predict some of these *rovA* regulated genes will be unique to *Y. pestis* (compared to *Y. enterocolitica* or *Salmonella*).

**Grant:** 1R21AI053303-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** BROOKMEYER, RONALD S PHD BIOSTATISTICS:PUBL  
HEALTH STATISTICS  
**Title:** Statistical Models for Anthrax  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): The objective of this application is to develop statistical models for anthrax outbreaks and the pathogenesis of inhalation anthrax. Because of limited laboratory and field study data on inhalation anthrax relative to other diseases, it is imperative to glean as much information as possible from the available data through development and application of state-of-the-art statistical methods and mathematical and statistical models. There are three specific aims: 1. Develop statistical models for anthrax outbreaks. These models would allow one to estimate the size of an anthrax outbreak, the impact of antimicrobial prophylaxis, the probability that an outbreak has ended, and the likely date cases were exposed to the spores. The methods will be applied to the 2001 U.S anthrax outbreak. The performance and operating characteristics of the methods will be evaluated by computer simulation. The methods will be extended to develop statistical algorithms that could be used to forecast and characterize future anthrax outbreaks. 2. Develop statistical and mathematical models for the incubation period distribution of inhalation anthrax that account for dependence on age and numbers of inhaled spores. These models will be based on reanalysis of the Sverdlovsk outbreak in 1979 as well as other published experimental and field studies. 3. Perform sensitivity analyses to underlying assumptions of the proposed models and methods, and, quantify the statistical error in the results. This work will address sensitivity of the results to the parametric form of the incubation period distribution, confidence interval procedures for parameters, and impact of phenomenon such as resuspension of spores or prolonged spore circulation in the atmosphere. This work will depend on results from specific aims 1 and 2.

**Grant:** 1R21AI053306-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** ZOLLA-PAZNER, SUSAN B. PHD  
MICROBIOLOGY:IMMUNOLOGY  
**Title:** Human Monoclonal Antitoxin to Anthrax Protective Antigen  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY  
**Project Period:** 2002/09/15-2004/08/31

DESCRIPTION (provided by applicant): Data from several animal models, as well as anecdotal evidence in humans, suggest that antibodies (Abs) to the protective antigen (PA) of anthrax are protective if administered in a timely manner. Unfortunately, a large and stable source of such Abs is not available to stockpile for use in case of a bioterrorist attack with *Bacillus anthracis*; however, cell lines making anti-PA monoclonal antibodies (mAbs) would provide a ready source for such reagents. While rodent anti-PA mAbs have been described, none has shown protection in animal models against fully virulent strains, although one did lead to a significant delay in guinea pigs in time to death. These negative results may be attributable to the use of mAbs specific for epitopes that do not neutralize the toxins and/or to the need to use a combination of two or more anti-toxin mAbs that would act synergistically to protect against the lethal effects of anthrax toxins. It is also possible that the mouse B cell repertoire against PA does not sustain a strong response against epitopes that are involved in toxin neutralization. Production and use of human monoclonal antitoxins would have many advantages: they are, by definition, completely human and are therefore usable without engineering as therapeutic reagents in humans. Moreover, they are representative of the protective human polyclonal Ab response against anthrax and would therefore provide information about the epitopes and Ab mimotopes that could be used to develop molecular vaccines, which would be more efficacious than those currently in use or in development. For these reasons, we propose experiments to produce and characterize human mAbs against PA of anthrax. Thus, in Aim 1 we propose to produce heterohybridomas making human anti-PA mAbs. These will be produced from the peripheral blood of human volunteers who are recipients of an experimental vaccine consisting of recombinant PA (rPA). Cells from vaccinees will be obtained ~1 week after the final immunization, transformed with Epstein-Barr virus, cultured and screened for anti-PA production. Cells in Ab-positive wells will be fused with a heteromyeloma line, repeatedly screened for reactivity, and cloned at limiting dilution until monoclonality is achieved. In Aim 2, the various mAbs made will be studied to determine the epitopes, which they recognize, and how specificity, affinity and IgG subtype correlate with protective activity. Protective functions will be assessed in vitro by measuring the ability of individual mAbs or combinations of mAbs to block the lethal effects of anthrax toxin.



**Grant:** 1R21AI053317-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KRULWICH, TERRY A PHD  
MICROBIOLOGY:BACTERIOLOGY  
**Title:** Bacillus anthracis Spores: Initiation of Germination  
**Institution:** MOUNT SINAI SCHOOL OF MEDICINE OF NEW YORK, NY  
NYU  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): An essential early step in the initiation of anthrax in the mammalian host is the germination of the Bacillus anthracis (endo)spores that have been engulfed by macrophages. The biochemistry of early steps in Bacillus spore germination is not yet understood. Current therapeutic interventions in the anthrax infectious cycle target much later steps. The proposed studies will extend promising preliminary findings on the three-protein GerB germinant receptor of Bacillus subtilis to the GerX and Gerl homologues of B. anthracis. GerX is encoded by a virulence plasmid and is required for full virulence, and Gerl is one of the chromosomally encoded receptors that may also contribute to germination in the macrophage. Like GerB, GerX and Gerl are homologous members of the GerA-type germinant receptors required for germination of Bacillus spores in response to specific nutrient germinants. Mechanisms for these important complexes will be sought. Preliminary evidence indicates that the GerB is an ion channel that is regulated by its nutrient germinants. The hypothesis underlying the application is: (i) that other GerA-type receptors also are either potassium or sodium channels; and (ii) when triggered by a germinant, ion flux through the receptor channel activates downstream ion transporters and other germination-related proteins leading to a cascade of germination events. Specific Aim 1 is to further characterize the biophysical, inhibition and regulatory properties of the GerB channel in the heterologous systems (Escherichia coli membrane vesicles and human embryo kidney cells) used in preliminary studies. The roles of the three GerB proteins will be further defined. Specific Aim 2 is to apply the same approaches to define the GerX and Gerl ion channel properties, germinants, inhibitors, and response to signals of the phagolysosomal environment. Single protein components of these complexes that exhibit channel activity will also be characterized. Specific Aim 3 is to identify candidates for the most proximal, "downstream" transporter(s) of GerX and Gerl and to characterize their activities and the basis whereby specific germinant receptors may activate specific downstream transporters. Such transporters may be new virulence factors, i.e. additional potential therapeutic targets.

**Grant:** 1R21AI053319-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** FETHERSTON, JACQUELINE D PHD  
**Title:** Identifying virulence factors in pneumonic plague  
**Institution:** UNIVERSITY OF KENTUCKY LEXINGTON, KY  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): *Yersinia pestis*, the causative agent of pneumonic and bubonic plague, is considered a potential bioterrorism agent. The bacterium is easy to grow and genetically manipulate and can be delivered in aerosolized droplets. The resulting pneumonic plague has a short incubation time and is rapidly and highly fatal. Great potential exists for spread of pneumonic plague from primary infected individuals to their contacts. Currently, there is no available vaccine against pneumonic plague. While much is known concerning the virulence determinants necessary for *Y. pestis* to cause disease by subcutaneous and intravenous routes of infection, essentially nothing is known about the virulence factors required for pneumonic plague. Such factors would be potential candidates for a subunit vaccine or targets for therapeutic agents designed to protect against pneumonic plague. Therefore the overall goal of this research application is to identify determinants important for the virulence of *Y. pestis* in a mouse model of pneumonic plague. The pH6 antigen is a putative adhesin, which is required for full virulence of *Y. pestis* by an intravenous route of infection, while different iron transport systems are important in the pathogenesis of bubonic and septicemic plague in mice. Recently we have discovered that a heme transport system, Hmu, is necessary for the optimal growth of *Y. pestis* in a macrophage cell line. In Specific Aim 1, we will use *Y. pestis* strains carrying specific mutations in the pH6 antigen and select iron/heme transport systems to test the potential role of these systems in the virulence of pneumonic plague. In Specific Aim 2, signature-tagged mutagenesis will be used to identify additional gene products required for the pathogenesis of pneumonic plague. These studies will enhance our understanding of the factors important in the pathogenesis of pneumonic plague and facilitate the development of measures to protect people against possible bioterrorism based on *Y. pestis*.

**Grant:** 1R21AI053360-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** MADDOCK, JANINE R PHD  
**Title:** Proteomics of B anthracis membrane and spore proteins  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): One of the requirements for accurate bio-detection of harmful pathogens is having a molecular profile of the organism of interest that is distinct from related, nonpathogenic species. A particularly difficult challenge has been the identification of species-specific membrane and spore coat proteins. My laboratory has made tremendous progress in optimizing the separation of both bacterial membrane proteins and spore coat proteins on 2D gels. This improvement in separation, coupled with the availability of genome sequence and recent advances in protein identification using peptide mass fingerprinting through mass spectrometry has made identification of these protein spots on a 2-D gels a reality. In this study we will perform a comprehensive proteomic analysis of the Bacillus anthracis membrane and spore coat proteins. From these studies we will have in hand an accurate catalog of expressed gene products during vegetative growth, during sporulation and of the mature spore coat. The identification of species-specific spore coat proteins is of particular necessity as the bioterrorism threat is specific to the spore form of B. anthracis. Our studies will identify the spore coat proteins that are unique to B. anthracis such that an accurate bio-detection assay can be rapidly developed. The data generated from this study will also allow us to conduct long-term studies that focus on molecular/genetic approaches to identify antimicrobial targets and to address basic developmental questions.

**Grant:** 1R21AI053365-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** DRIKS, ADAM PHD  
**Title:** IDENTIFICATION OF B. ANTHRACIS SPORE-SURFACE PROTEINS  
**Institution:** LOYOLA UNIVERSITY CHICAGO MAYWOOD, IL  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): As current events dramatically indicate, reliable accurate and portable *Bacillus anthracis* spore detection systems are critical for national defense and civilian health. In an effort to meet this need, several candidate technologies are under examination by a number of federal agencies. Detectors using the outermost proteins of the spore coat as signature molecules for identification would represent a particularly compelling technology. This approach is not currently feasible, as the identities of the *B. anthracis* surface proteins are unknown. However, based on the completed *B. anthracis* genome sequence and extensive analysis of the related organism *B. subtilis*, we can now identify many spore-surface protein candidates. In addition, a variety of approaches to identify these proteins de novo are available. Therefore, we propose using a combination of biochemical and genetic methods to test candidate proteins for a surface location as well as to identify these proteins directly. Our approaches take advantage of proteomic analysis of the *B. anthracis* and *B. subtilis* coats ongoing in our laboratory, as well as the available genome sequences of the two organisms. Ultimately, this work will enable development of a new class of small, robust versatile *B. anthracis* spore detectors.

**Grant:** 1R21AI053369-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** WANG, JULIA Y PHD  
**Title:** DEVELOPMENT OF MULTIVALENT ANTHRAX TOXIN INHIBITORS  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2002/09/15-2004/08/31

DESCRIPTION (provided by applicant): As demonstrated so terribly by recent events, anthrax poses a deadly threat as a biological weapon of terrorism and warfare. Systemic intoxication by anthrax toxins is virtually always fatal but effective treatment is not available. The goal of this application is the development of potent inhibitors that prevent the assembly of anthrax toxin complexes. Anthrax toxins are responsible for the major symptoms of the disease. The toxins consist of a host receptor-binding protein termed protective antigen (PA) and two enzymes termed lethal factor (LF) and edema factor (EF). These proteins are released from *Bacillus anthracis* as nontoxic monomers. They diffuse to the surface of host cells and assemble into two types of toxic protein complexes, lethal toxin (LF+PA) and edema toxin (EF+PA). PA is the necessary vehicle that transports LF and EF from the cell surface to the cytosol where LF and EF exert their cytotoxic effects. Hence, inhibitors that prevent the binding of LF or EF to PA should provide an effective antitoxic therapy against anthrax. Previously, we have identified several peptides that bind PA weakly and inhibit the interactions of LF or EF with PA. We hypothesize that, through cooperative interactions, multivalent inhibitors (MVIs), in which multiple copies of a peptide are coupled to a carrier molecule, will display significantly enhanced inhibitory effects. In our preliminary study, we have synthesized several dextran-based MVIs that show higher inhibitory activities than peptides alone. We propose to develop optimized MVIs based on biocompatible, nontoxic, linear polymers and cyclic oligomers as carriers. Aim 1. To optimize MVIs based on dextran and pectin carriers. Aim 2. To explore MVIs based on polyglutamate backbones. Aim 3. To design and develop heptavalent "crown" MVIs based on (-cyclodextrin cores. In Aims 1 and 2, we plan to synthesize a series of peptide-polymer conjugates in which the peptide-to-backbone ratio and the molecular size of the backbone are systematically varied. In Aim 3, we will assist the design of crown inhibitors by computational modeling. We will test all MVIs in our established inhibition assays and the most active MVIs in two rat intoxication models. Potent inhibitors developed in this study can help to protect us from deadly anthrax disease and fight the threat of anthrax bioterrorism.

**Grant:** 1R21AI053376-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KLEMPNER, MARK S MD INTERNAL  
MED:INFECTIOUS DISEASE  
**Title:** New Method for Detecting Bacillus Anthracis Spores  
**Institution:** BOSTON MEDICAL CENTER BOSTON, MA  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): The threat of attack on military and civilian targets with biological weapons is a growing national concern and the development of rapid, high-sensitivity, high-accuracy point detection systems for detecting and identifying bioagents in the environment and in biological specimens is a critical requirement. We will focus this effort on a new, broadly applicable method for the detection of B. anthracis spores. Currently available methods for detecting and identifying bioagents provide high sensitivity detection and highly selective identification, but the time required to chemically amplify detectable components and the size/power/cost requirement for sensor readout hardware make current methods unsuitable for point detector systems that can be placed onto any platform (including personnel or unmanned vehicles), dispersed, remotely placed, and networked. We propose to develop a revolutionary method for detecting and identifying bioagents that provides a detailed signature for a biological pathogen in real time without the use of chemical amplification. Our method relies upon measuring the affinity of bioagent outer surface components to a library of ligands immobilized onto a novel biosensor chip. Proof-of-concept of this strategy for detection, identification and discrimination of fungal spores has been demonstrated as part of a NASA funded program for the detection of microorganisms in the environment of manned space vehicles for exploratory space travel. A microarray affinity detection system that relies on both positive affinity of the agent for defined ligands as well as the lack of affinity for a separate set of ligands allows a high degree of redundancy for the specific identification of the agent as well as a powerful technology to avoid "false alarms". The sensor approach utilizes a colorimetric resonant reflectance biosensor that will allow detection hardware to be miniature, low cost, low power, and rugged. Bioagent identification is performed through automated analysis of the recognized outer surface ligands that can classify detected material (spore/bacterium/protein, alive/dead, toxic/nontoxic, pathogen family). The use of a large integrated biosensor array will provide a highly detailed bioagent signature for accurate identification with sufficient sensor redundancy to minimize false alarms. Differential signature response will be demonstrated by exposure of the microarray to vaccine strain B. anthracis spores and spores of other related and unrelated bacteria. The project will focus on developing the ligand library to include non-antibody reagents and differential recognition of bacterial spores from multiple species.

**Grant:** 1R21AI053389-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** MARKS, JAMES D MD  
**Title:** Deciphering toxin neutralization by oligoclonal antibody  
**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): BoNTs are classified as one of the six highest-risk threat agents for bioterrorism (the 'Class A agents'). BoNTs have been produced and weaponized by rogue nations and deployed by terrorist groups. As a result, specific pharmaceuticals are needed for prevention and treatment of intoxication. The goal of this application is to generate a novel Ab that can be used to prevent and treat disease caused by BoNT/A. This application builds on work showing that BoNT/A can be potently neutralized in vivo by combining three mAbs, which recognize the toxin domain (Hc), which binds cellular receptors (oligoclonal Ab). Toxin neutralization by oligoclonal Ab results from a large increase in the affinity of Ab for toxin as well as progressive blockade of the toxin surface interacting with cellular receptors. The precise contribution of these two mechanisms to toxin neutralization is unknown, as is the requirement for mAbs to the toxin-binding domain vs. mAbs to other parts of the toxin. Two important questions remain prior to producing a BoNT/A Ab therapeutic: 1) Can the potency of oligoclonal Ab be reproduced in a single mAb (or mAb pair) by significantly increasing the affinity of the mAbs?; and 2) can a similar potency be achieved using mAbs to non-binding domains of the toxin? Reducing the number of mAbs would greatly simplify the complexity and cost of Ab manufacturing. Using non-binding domain mAbs would demonstrate that potent toxin neutralization does not require mAb binding toxin epitopes that interact with cellular receptors. This would simplify identification of neutralizing Abs. In addition, such mAbs would allow neutralization of second generation BoNTs, where the binding domain has been replaced with a receptor ligand. To determine the impact of affinity on BoNT/A neutralization, the affinity of two mAbs, which bind BoNT/A and neutralize toxin in vitro, will be increased at least 100 fold using in vitro mutagenesis and selection. The impact of affinity on in vitro and in vivo toxin neutralization will be determined for the single mAbs, a combination of the mAb pairs, and oligoclonal Abs. To determine the ability of non-binding domain BoNT/A Abs to neutralize toxin, phage Abs recognizing non-binding domain portions of BoNT/A will be generated and characterized with respect to affinity, epitope, and ability to neutralize toxin in vitro and in vivo. In vitro and in vivo characterization will be performed on individual mAbs, as well as combinations of binding domain and non-binding domain mAbs. Answering the above questions will generate a pharmaceutical for prevention and treatment of BoNT/A disease and also provide a route to similar pharmaceuticals for other BoNT serotypes. In addition, this approach would be applicable to four of the other Class A agents (anthrax, smallpox, plague, and hemorrhagic fever viruses).

**Grant:** 1R21AI053397-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** O'BRIEN, ALISON D PHD  
MICROBIOLOGY:MICROBIOLOGY-UNSPEC  
**Title:** Immunoprotective monoclonals to B anthracis spores  
**Institution:** HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD  
MIL/MED  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): Bacillus anthracis spores were recently used as agents of bioterrorism. Among the many negative consequences of these deliberate instances of microbiological sabotage was one positive outcome: not all of the 11 victims with the typically lethal inhalational form of anthrax died. Indeed, the aggressive use of quinolones and other antibiotics coupled with the early recognition of disease resulted in the survival of 6 of the 11 patients. Unfortunately, hundreds of other individuals potentially exposed to the anthrax spores required an extended course of antibiotic therapy. A remaining health concern is that the people who received antibiotic prophylaxis may still present with inhalation anthrax after conclusion of their therapy as dormant viable spores germinate. One way to increase the likelihood that patients with disease will survive and that those exposed will have a higher probability of remaining healthy is to prevent the infectious dormant spores from germinating and subsequently transforming to vegetative cells. Recent evidence that antibodies against the PA (the shared B subunit for the two A subunit toxins of B. anthracis, edema factor and lethal factor) actually bind to the surface of spores and decrease the level of spore germination, taken with the fact that formaldehyde-inactivated spores can serve as a protective vaccine against anthrax in guinea pigs, led us to the following hypothesis: mAbs against PA and/or other spore-surface-expressed antigens can block spore germination or render spores more susceptible to phagocytosis and ultimately killing by macrophages. Based on this theory, our goals are to develop immunoprotective mAbs against B. anthracis spores that confer protection against anthrax in animal models. Ultimately, we intend to humanize those mAbs for use as short-term preventative agents or therapeutic modalities. The Specific Aims are as follows. Specific Aim 1 is to elicit mouse antisera against irradiated B. anthracis dormant spores, irradiated activated spores, activated spore-surface protein extracts, and recombinant PA (rPA). We will test those antisera in enzyme-linked immunosorbent assays (ELISAs), in vitro germination assays, and macrophage assays to evaluate phagocytosis, germination, and sporicidal activity. We will then prepare mAbs from those animals whose sera demonstrate one or more of these anti-spore related activities. Specific Aim 2 is to assess the capacity of these B. anthracis mAbs to prevent anthrax disease first in a mouse parenteral spore challenge model and, if protective, in a rabbit model of inhalational anthrax. Specific Aim 3 is to identify the spore-surface protein(s) recognized by each mAb through N-terminal sequencing. Specific Aim 4 is to initiate, with SUNOL Molecular Corporation, the engineering of humanized versions of those mAbs that confer protection.



Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R21AI053403-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** HORWITZ, MARCUS A MD  
**Title:** Characterization of the Francisella tularensis phagosome  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2002/09/15-2004/08/31

DESCRIPTION (provided by applicant): Francisella tularensis is a facultative intracellular bacterial pathogen that causes serious and potentially life threatening illness. Because the bacterium grows readily in broth culture, has extraordinarily high infectivity, causes serious morbidity and mortality, and is easily dispersed, it is also considered a potential agent of bioterrorism. While currently available antibiotics are effective in treating tularemia, F. tularensis can be engineered to carry antibiotic resistance genes. For these reasons, new approaches to treatment and prevention of tularemia are needed. However, devising such strategies requires an improved understanding of the cell biology of F. tularensis. At present, very little is known about the interactions between F. tularensis and its host cells or of the pathogenic mechanisms that allow F. tularensis to enter, survive, and multiply within host cells. The main objective of this application is to improve our understanding of the cell biology of F. tularensis. Specifically, our aims are: (a) to examine the adherence, uptake and growth of F. tularensis in human mononuclear phagocytes to confirm it as a model system for further study; (b) to examine the ultrastructural features of the F. tularensis compartment in human macrophages at sequential times after infection; and (c) to define the membrane trafficking interactions of the host cell and the F. tularensis compartment by immunofluorescence and immunoelectron microscopy and by examining the effect of overexpression of wild-type and mutant proteins involved in membrane trafficking. We shall combine techniques of cell biology, molecular biology, and immunoelectron microscopy to accomplish these research objectives. An improved understanding the cell biology of F. tularensis is the first step in understanding its pathogenic mechanisms. Understanding how F. tularensis subverts the host cell membrane trafficking pathways and attains an intracellular compartment that is hospitable for its survival and growth will help guide new strategies for the prevention and treatment of tularemia.

**Grant:** 1R21AI053410-01

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

**Principal Investigator:** VOGT, PETER K

PHD ZOOLOGY

NEC:ZOOLOGY NEC-UNSP

**Title:** Potent inhibitors of Anthrax Lethal Factor

**Institution:** SCRIPPS RESEARCH INSTITUTE

LA JOLLA, CA

**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): The aim of this application is to isolate potent inhibitors of the lethal factor (LF) metalloprotease of *Bacillus anthracis*. We will develop a high throughput fluorescence-based assay for LF, starting with the natural target peptide derived from MAP kinase kinase and optimizing the reaction by developing and selecting variants of the natural substrate. The assay will be used to screen combinatorial chemical libraries and "click" chemical libraries. The basic approach of click chemistry is to design two ligands that will become covalently linked upon binding to the substrate. This linkage reaction will be the 1,3-dipolar cyclo-addition of azides and acetylenes. Preliminary work using this approach on acetylcholinesterase has yielded an inhibitor with a KD of 100 fM. Click chemistry will be complemented by traditional combinatorial chemistry. Libraries will be targeted using the structural information available for LF, supplemented by information from point mutations of the substrate. Information from our previous synthesis of metalloprotease inhibitors will also be used.

**Grant:** 1R21AI053426-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** ARTENSTEIN, ANDREW W MD  
**Title:** Novel Approaches to the Inhibition of Anthrax Toxin  
**Institution:** MEMORIAL HOSPITAL OF RHODE ISLAND PAWTUCKET, RI  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): *B. anthracis* is considered to be among the most attractive agents for use in bioterrorism due to its clinical virulence. It has recently been deployed as a weapon in the U.S. with an attendant 45% mortality, despite the aggressive use of potent antimicrobials. Novel approaches to the therapy of anthrax are needed. *B. anthracis* exerts its pathogenic effects primarily through the action of lethal toxin, a combination of two proteins elaborated by the organism after infection: protective antigen and lethal factor. Because the synthesis of *B. anthracis* toxin is dependent on receptor binding and subsequent activation by a specific serine protease, the use of a receptor antagonist, von Willebrand factor concentrate, and the protease inhibitors antithrombin-III and inter-alpha-inhibitor, may prevent initial intoxication. Interleukin-11 may prove useful at subsequent steps to inhibit toxin action by attenuating the pro-inflammatory cytokine response. These potential therapies are all endogenous proteins and have the advantage of favorable clinical track records and safety profiles when used for other indications. Most are licensed for use in humans and therefore clinically available. The effects of these interventions on the action of *B. anthracis* toxin will be investigated initially in murine peritoneal macrophages, a system generally susceptible to the lethal effects of this toxin. Measurements of inflammatory cytokines and cell viability assays will be used to assess the impact of the therapeutic interventions on the activity of *B. anthracis* toxin. The importance of the temporal delivery of toxin inhibition strategies will be determined by varying the timing of therapy relative to intoxication. The *in vivo* effects of these therapies will be studied in mice with survival as an end-point. If successful, this research will lead to future, collaborative studies involving animal challenges to determine whether toxin inhibitors will provide additive or synergistic benefits when used in conjunction with existing antimicrobial approaches for the therapy of anthrax. Since antithrombin-III, interleukin-11 and von Willebrand factor are already approved for human use for other indications, it should be possible to rapidly move into clinical trials with the optimal combinations of agents as determined in these pre-clinical studies.

**Grant:** 1R21AI053432-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** STEBBINS, C E PHD  
**Title:** Novel Plague Antibacterials Through Phage Display  
**Institution:** ROCKEFELLER UNIVERSITY NEW YORK, NY  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Biowarfare agents, like chemical weapons, have often been termed the "poor man's atomic bomb," because of their potential for devastation and the relative ease with which they can be weaponized at low cost. *Yersinia pestis* is an infectious agent of particular concern because it has been very effectively weaponized and causes debilitating and often fatal illnesses -bubonic and pneumonic plague. While antibiotic treatments exist to counter biological attack with this agent, there are serious drawbacks with the available options. Strains of plague have already arisen naturally, which are resistant to most antibiotic compounds. Antibiotics to combat plague can also be countered easily by genetically engineered strains, which harbor resistance genes to these compounds. It is likely that this has been extensively pursued in the former Soviet bioweapons program. It is therefore a matter of public health and potentially national security to protect the population from the possible use of this agent. One way to do this is, to develop a new class of therapeutic compounds that can be used to treat an exposed or ailing population. In particular, developing drugs that inhibit virulence mechanisms of this pathogen instead of targeting vital processes would represent a novel class of antibacterial with many advantages over existing therapies. The overall goal of this application is to test the efficacy of using phage display peptide technologies to identify novel drugs that impair the virulence mechanisms of plague bacterium. Specifically, we wish to: (1) use phage display to identify high affinity peptide binders for molecules central to the virulence system of *Y. pestis*, (2) test these peptides for their ability to impair biochemical function of the pathogen's proteins and (3) use structural biology to examine the nature of the inhibitor-protein interactions in order to improve by rational design the biological activity of the peptides. These studies are therefore intended as a pilot experiment, which would lead to a broader initiative to examine the efficacy of these inhibitors in vivo, and to bring them to the drug development stage. The threats of biological attack require that a variety of therapies be available as countermeasures. These studies may lead to the discovery of novel classes of antimicrobial compounds, which may serve to combat these emerging threats.

**Grant:** 1R21AI053444-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KONG, FAN-KUN MD  
**Title:** Modified adenovirus with selective tropism in Langerhans  
**Institution:** VAXIN INC. BIRMINGHAM, AL  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Immunization through topical application without physical penetration by needles has been proved to be effective in eliciting protective immune responses. This novel vaccination modality could potentially immunize a large population of diversified age and health status within a short timeframe during a bioterrorism attack. We are developing a skin-applied, adenovirus (Ad)-vectored anthrax vaccine. One of the problems encountered in using Ad as the vector for skin-applied DNA vaccines is its low efficiency in transducing antigen presenting cells. Ad infection depends on the expression of Coxsackie-adenovirus receptor (CAR) on the surface of its target cells. The limited expression of CAR by Langerhans cells (LC), the principal antigen capture cells in the skin, greatly hindered Ad vector entry and minimized the encoded antigen gene expression. We hypothesize that modifying Ad vector with enhanced tropism for LC could facilitate Ad entry into LC and greatly increase the antigen gene expression, and thereby more effectively mobilize the immune system and amplify the magnitude of host immune responses. Ad vectors with selective tropism for LC will be developed by coating the Ad fiber with LC-targeted ligands. Potency of the new vector will be rigorously examined by inoculation via both a patch and a syringe needle, and compared to that of a spore-based anthrax vaccine.

**Grant:** 1R21AI053508-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** GARCIA, EMILIO PHD  
**Title:** Unique Genomic Regions of *Y. pestis* in Pathogenesis  
**Institution:** UNIVERSITY OF CALIF-LAWRNC LVRMR LIVERMORE, CA  
NAT LAB  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): *Yersinia pestis*, the etiological agent of the acute diseases bubonic and pneumonic plague, has been one of the most devastating epidemic-causing bacteria experienced by mankind. Although plague is not a public health problem in most parts of the world, its potential for contagion, the lack of an effective vaccine, and the recent emergence of multiple antibiotic resistant strains place this organism squarely at the top of the United States' select agent list as a potential candidate for bioterrorism use. The long-term goal of this application is to elucidate the molecular mechanisms that underlie the nature of the acute bacterial infectious process in *Y. pestis*. The more immediate objective of this work is to identify novel virulence genes that will serve as targets in the development of robust diagnostics assays (especially for genetically-engineered organisms) and of non-antibiotic therapeutics. Specific aims of this application are as follows. Specific Aim 1 is to determine the role played by *Y. pestis*-specific genomic regions in its pathogenicity by generating systematic knockouts of these genomic regions. These unique regions will be identified by direct, whole-genome comparison between *Y. pestis* and its enteropathogen progenitor, *Y. pseudotuberculosis*, while the putative change in virulence will be determined using a murine model of infection. Specific Aim 2 is to evaluate the contribution to virulence of *Y. pestis* genes differentially expressed in relation to its near-neighbor, *Y. pseudotuberculosis*. This will be done by first identifying the differentially-expressed genes using an available whole-genome microarray of the two *Yersinae* spp. followed by generation of knockouts as described for Specific Aim 1. Specific Aim 3 is to characterize the genome-wide expression profile of attenuated mutants obtained in Specific Aims 1 and 2 in order to identify the relevant virulence pathways of this organism. This will be achieved using similar microarray analyses. The outlined application will serve as an important first step in elucidating the genomic basis for the dramatically different clinical manifestations of these two genetically related pathogens. This work will provide a unique model for defining the nature of this and other acute infectious diseases through the use of recent advances in comparative and functional genomics. Similar approaches can be used to study the virulence of other potential bacterial bioterrorism agents.

**Grant:** 1R21AI053517-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** LINDBERG, IRIS  
**Title:** Blockade of Anthrax Cytotoxicity Using Furin Inhibitors  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS, LA  
ORLEANS  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): The anthrax bacillus produces a three-component exotoxin of which an essential element for bioactivity is the protein known as protective antigen, or PA. PA binds to a cell surface receptor and is cleaved to generate a 63 kDa protein to which the one of the other anthrax toxins, LF and EF, can bind. Proteolysis and binding of PA permits internalization of a PA-LF complex into the cytosol, where it is able to attack cellular machinery, resulting in cell death. Since proteolytic cleavage of the PA anthrax toxin is obligatory for the manifestation of toxic activity, this cleavage step represents a natural target for pharmacologic intervention. Previous research has shown that this cleavage is performed by a cellular surface enzyme known as furin, a member of the family of eukaryotic subtilisins. This application is directed toward the idea that the cytotoxic action of anthrax toxin can be attenuated through inhibition of the activating cleavage event, resulting in lessened toxicity and cellular protection. In the last decade, several groups have shown that it is possible to block cleavage of bacterial toxins using engineered protein inhibitors of furin. We have recently used combinatorial chemistry techniques to identify a stable hexapeptide, D6R {D-hexa-arginine) which represents a potent, stable small molecule inhibitor of furin. Our preliminary data indicate that D6R can effectively inhibit furin-mediated cleavage of a bacterial toxin derived from *Pseudomonas*, blocking lethal effects in both cell lines as well as live animals. We here propose to systematically apply our studies of furin inhibition to the blockade of anthrax toxin activation. Specifically, we will investigate the use of D6R itself as a potential therapeutic in the attenuation of anthrax toxin cytotoxicity; examine the structural requirements for inhibition of furin-mediated cytotoxicity by D6R related molecules; and test D6R and/or other stable furin inhibitors identified in this work in animal models of anthrax toxicity. Our preliminary data showing potent inhibition of PA cytotoxicity by D6R support the idea that small molecule furin inhibitors will represent effective agents for the biologic attenuation of anthrax toxin cytotoxicity, the development of such antitoxin agents will add significantly to our ability to protect organisms against this pathogen.



Date Run: 04/26/05

NIH Extramural Support in Bacteriology Research

**Grant:** 1R21AI053521-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** YANG, DAVID C  
**Title:** Novel recombinant protein therapeutics of botulism  
**Institution:** GEORGETOWN UNIVERSITY WASHINGTON, DC  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): Botulinum toxins are among the most deadly biological agents with the capacity of mass destruction. The tripartite toxin consists of a receptor binding domain, a membrane translocation domain and a proteolytic catalytic domain. Botulinum toxins bind specifically to synapses at the neuromuscular junctions by the receptor binding domain, penetrate the nerve cells by the translocation domain, destroy secretory protein assembly by the catalytic domain, and result in paralysis and possible death. No therapeutic drugs are currently available to treat affected individuals. In view of notable recent successes of peptide and recombinant protein therapeutics of anthrax, breast cancer, arthritis, etc., the objective of the proposed project is to develop recombinant protein drugs for botulism. The Specific Aims for the granting period will be first isolating peptides that will not only specifically inhibit the proteolysis but will also rescue damaged nerve cells and, secondly, to develop a targeting and delivery system for such peptides. Peptides that bind the catalytic domain will be initially isolated through combinatorial phage display peptide libraries based on structures of existing inhibitors as well as random search. A high throughput assay of the protease activity in botulinum toxins will then be used to identify inhibitory peptides. Further modifications and expansion of the inhibitory peptide structure will be made to improve the affinity and the specificity of the peptides. Peptides that rescue damaged cells from the toxicity will be selected. Recombinant proteins will then be synthesized that 1) have identical synaptic binding properties as botulinum toxins; 2) contain the intact translocation domain; 3) contain an inactive proteolytic catalytic domain; and 4) fuse with toxin inhibitors, neutralizing proteins, and rescue peptides. The effectiveness of the recombinant proteins in neutralizing and inhibiting botulinum toxin will be examined in vitro and in vivo. Such recombinant proteins are expected to bind to the same target nerve cells as botulinum toxins, to translocate the catalytic domain across the membranes, and eventually to specifically inhibit the degradation of synaptic proteins by the toxins and rescue damaged cells in vivo. The

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

results could be readily applicable to other toxins of the same family and helpful for vaccine development.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R21AI053523-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** SIEBURTH, SCOTT M PHD ORGANIC CHEMISTRY  
**Title:** Anthrax LF Inhibitor Drug Based On Organosilanes  
**Institution:** TEMPLE UNIVERSITY PHILADELPHIA, PA  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Silanediols are novel transition-state analog protease inhibitors pioneered in the Principal Investigator's laboratory. These structures are effective inhibitors of metalloprotease enzymes at low nanomolar (nM) levels and can cross cell membranes at rates comparable to non-silane (commercial) pharmaceuticals. B. anthracis LF is a metalloprotease and the source of anthrax toxicity. No effective inhibitor of this enzyme has been described. The initial phase of this research will produce a silanediol inhibitor of LF using known substrate specificities. The second phase will structurally alter this inhibitor to remove the peptide character and thereby improve pharmacokinetic properties. Structural fine-tuning will then yield a clinical candidate for victims of systemic anthrax infection who cannot be saved by antibiotics. -  
Pro-Val-Leu-Pro-Ala-Leu-Thr- LF substrate Phase 2 and  
site of cleavage

**Grant:** 1R21AI053524-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** SIMON, SANFORD R PHD  
**Title:** Treatment of Anthrax with Nonantimicrobial Tetracyclines  
**Institution:** STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): We propose a pharmacologic strategy to combat the effects of LeTx of *Bacillus anthracis*, the cause of anthrax, based on the pleiotropic actions of non-antimicrobial CMTs. The CMTs are inhibitors of zinc matrix metalloproteases (MMPs), but they also can inhibit zinc metalloproteases of bacterial origin. Lethal Factor (LeF), the proteolytic subunit of LeTx, is a zinc metalloprotease with a catalytic domain similar to thermolysin and is a potential target of the CMTs as well. The human cell most sensitive to LeTx is the macrophage, which is activated through proteolysis of multiple MAPK kinases (MAPKKs) to trigger a systemic inflammatory response that may develop potentially fatal pulmonary complications of Acute Respiratory Distress Syndrome (ARDS). We have shown that, in addition to their inhibition of MMPs, the CMTs downregulate responses of activated macrophages, including release of MMPs and generation of nitric oxide (NO). With collaborators at SUNY Syracuse, we have shown that in multiple porcine models of ARDS, administration of CMT-3 prevents the otherwise fatal progression of lung damage while leukocyte respiratory burst activity, leukocyte infiltration of the lung alveolar spaces, and levels of leukocyte-derived proteases are all diminished. In vitro treatment with CMT-3 of the blood from human patients whose leukocytes were activated in vivo also results in a diminished leukocyte respiratory burst. We therefore propose to evaluate the inhibitory potency of CMT-3 and CMT-308 (less cytotoxic and photosensitizing than CMT-3) against the proteolytic activity of purified LeF, initially using a novel fluorogenic synthetic oligopeptide substrate specific for this metalloprotease. We will then evaluate the capacity of the CMTs to inhibit LeF-mediated cleavage of the natural macrophage substrates Mek-1 and Mek-2, using human macrophage lysates as sources of the MAPKKs and detection by Western blotting. Finally, we will evaluate the capacity of the CMTs to suppress the inflammatory response of LeF-exposed human monocytes, monocyte-derived macrophages, and a human monocytoid cell line, Mono Mac 6, by introducing LeF alone directly into the macrophage cytosol with a protein transfection agent (Chariot(tm), Active Motif Corp.). We will measure release of reactive oxygen species by DGFH oxidation, NO release with ozone chemiluminescence, secretion of MMPs by zymography with subsequent quantitation by ELISAs, and release of cytokines, also by ELISAs, as indicators of extent of LeF-triggered macrophage activation in the absence and presence of CMTs. Because GMT-3 has already been shown to be safe in Phase I trials with normal volunteers, if efficacy can be shown, it may potentially be rapidly deployed for protection in cases of possible anthrax exposure.

**Grant:** 1R21AI053526-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** COOK, JAMES L MD  
**Title:** Macrophage-Dependent Immunopathogenesis of Anthrax  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 2002/09/01-2004/08/31

Bacillus anthracis can induce a bacteremic phase of infection that is associated with a shock syndrome and a high-level rate of mortality. Most research in this field is focused on toxin production and function. Relatively little is known about the immunopathogenesis of the disease process related to macrophage infection, activation and sensitization to toxin-mediated triggering. Studies using other intracellular pathogens suggest that infection induced macrophage activation is an independent process from toxin-induced triggering of the shock syndrome. The working hypothesis for this proposal is that Bacillus anthracis induces a similar, 2-step process of macrophage activation followed by toxin triggering and that these are two, independent stages of macrophage- related immunopathogenesis of the shock syndrome. The objectives of this project are to establish an animal model of Bacillus anthracis-induced priming and toxin-induced triggering of the shock syndrome to test the hypothesis that these are independent processes. Contrasts will be made with the well-establish model of BCK-induced priming and endotoxin-induced triggering of the shock response. In vitro corollaries of infection-induced macrophage activation will test the phenotypic and functional changes of macrophage function following Bacillus anthracis infection in vivo and in vitro. Preliminary studies will test the linkage between infection-induced macrophage activation and triggering of the NF kappa B-dependent transcription response. Complementary in vitro studies will be used to test the prediction that toxin-induced triggering of activated macrophage populations will result in changes in toxin receptor expression, cytokine production and nitric oxide production and NF kappa B activation. The long-term objective is to develop an animal model and in vitro cell systems to test the functional relationships between Bacillus anthracis- induced macrophage activation and toxin-mediated triggering of activated macrophage functions that in part mediate the shock syndrome. This information will be used to seem molecular interventions that reduce or abrogate the shock response to this infection.

**Grant:** 1R21AI053528-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KARIN, MICHAEL PHD  
**Title:** How Anthrax lethal factor kills activated macrophages  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2002/09/15-2004/08/31

DESCRIPTION (provided by applicant): *Bacillus anthracis*, the causative agent of inhalation, cutaneous, and gastrointestinal anthrax, is an aggressive pathogen, whose unique properties make it an ideal bioterrorism agent. After inhalation, *B. anthracis* spores germinate in alveolar macrophages, which carry the bacteria to lymph nodes where they replicate and eventually disseminate through the blood stream. During this process, *B. anthracis* kills the macrophage and thereby evades detection and attack by the host innate immune system. Macrophage killing is mediated by the lethal toxin (LeTx), whose active subunit is lethal factor (LF). Curiously, at very low concentrations, LF activates the macrophage to produce proinflammatory mediators, whereas at higher concentrations it displays selective cytotoxicity toward macrophages. We found that modest concentrations of LF selectively induce the apoptosis of activated but not resting macrophages. This process is likely to depend on the ability of LF to cleave MAPK kinases (MKKs) at a site required for MAPK activation. Our results suggest that activation of p38 MAPKs together with the activation of NF- $\kappa$ B is necessary for prevention of activation-induced death of macrophages. We plan to test the hypothesis that LF specifically kills activated macrophages by targeting this anti-apoptotic mechanism. We also plan to better understand why at low concentrations LF activates, rather than kills, macrophages. We therefore plan to pursue the following specific aims: 1) Determine the signal transduction mechanism by which very low concentrations of LF induce macrophage activation and production of proinflammatory cytokines; 2) Determine the mechanisms by which modest concentrations of LF induce the apoptosis of activated macrophages; 3) Identify which NF- $\kappa$ B target genes protect activated macrophages against LF-induced apoptosis and which genes are induced in response to very low, non-cytotoxic, concentrations of LF to mediate inflammatory shock; and 4) Identify the mechanism for activation-induced apoptosis of macrophages that lack NF- $\kappa$ B or p38 activity. In addition to providing a much better molecular understanding of the interaction between *B. anthracis* and its host, this project should enable the design of new therapeutic strategies that will tilt the balance in favor of the host macrophage in the battle against *B. anthracis* and inhalation anthrax.

**Grant:** 1R21AI053652-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** SWANSON, JOEL A  
**Title:** Mechanisms of Anthrax Toxin Signaling in Macrophages  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): Macrophages are a primary cellular target of anthrax lethal toxin (LT), a bacterial secreted A-B toxin comprised of the proteins protective antigen (PA) and lethal factor (LF). PA and LF assemble on macrophage surfaces and are internalized by endocytosis. In a pH-dependent translocation process, PA delivers LF across endosomal membranes into macrophage cytosol where the protease activity of LF generates signals that lead to rapid death of macrophages, and possibly also to release of the inflammatory cytokines TNFalpha and IL-1beta. Experimental models of inflammation and bacterial infection have described a macrophage-specific, inflammatory cell death pathway, called pyroptosis that requires the protease caspase-1. Similarities between LF toxicity and pyroptosis lead to the hypothesis that cytosolic LF activates caspase-1 in macrophages, followed by cell death and release of the cytokines IL-1beta and IL-18. Addressing three specific aims will test this hypothesis: Aim 1. Define the roles of caspases in LF-mediated macrophage cytotoxicity and cytokine secretion. We will define the relationship between LF toxicity and caspase-1- dependent pyroptosis. Caspase activities will be measured in macrophages exposed to LF or ATP, or infected with *S. typhimurium*. LF-mediated cytotoxicity and cytokine release will be measured to determine the protective effects of specific caspase inhibitors and inhibitors of ATP-mediated cytotoxicity and cytokine secretion. Aim 2. Develop FRET-based probes of intracellular protease activities. An LF probe will be developed, consisting of cyan fluorescent protein (CFP) and citrine (a modified yellow fluorescent protein) linked by an LF protease substrate bridge. The intact molecule will exhibit fluorescence characteristic of FRET; and proteolytic cleavage of the bridge by LF will lead to loss of FRET. The LF probe will be purified for in vitro characterization, and will be expressed in macrophages for in situ characterization. It will then be used to define the timing of LF activity, relative to other physiological changes, in individual cells and populations of macrophages. To detect downstream signals generated by LF, analogous FRET -based probes of calcium and caspases-1, -3 or -8 will be developed, tested and employed. Aim 3. Define the temporal order of LF signals in sensitive and resistant macrophage. Using fluorescent probes for LF, calcium, and caspases, as well as biochemical assays for TNFalpha, IL-1beta, IL-18, reactive oxygen intermediates and cytotoxicity, we will determine the chronology of events associated with LF signaling in macrophage cytoplasm. The temporal order of signaling will also be defined by timed addition of signal inhibitors. Essential pathways leading to cytotoxicity will be defined by comparing the various LT responses in macrophages from sensitive and resistant strains of mice. These studies will lie out the essential pathway of LT- dependent macrophage cytotoxicity and address important outstanding questions about macrophage cytokine responses. In doing so, they will guide the selection and design of effective, late-stage therapeutics.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials



**Grant:** 1R21AI053759-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** BLISKA, JAMES B PHD  
**Title:** Microarray Analysis of Plague-Induced Apoptosis  
**Institution:** STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY  
BROOK  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): The plasmid-encoded type III secretion system of *Yersinia pestis* functions to deliver a set of toxins (Yops) into host eukaryotic cells. The Yop toxins modulate signaling pathways in host cells to neutralize innate immune mechanisms. The toxin YopJ triggers apoptosis in macrophages infected with *Yersinia*. YopJ is structurally related to a family of cysteine proteases, but the precise target(s) of its toxic activity remain unknown. It has been shown that YopJ inhibits several signaling pathways that are responsible for activation of transcription factors. The signaling pathway that activates the transcription factor NF- $\kappa$ B is a key target of YopJ. It is hypothesized that YopJ promotes macrophage death by reducing expression of one or more apoptosis inhibitor genes that are regulated by NF- $\kappa$ B. We will use microarray analysis to determine if any genes encoding apoptosis inhibitors are expressed at lower levels in macrophages infected with wild-type *Y. pestis* as compared to macrophages infected with YopJ *Y. pestis*. Apoptosis inhibitor genes that are down regulated in a YopJ-specific manner will be overexpressed in macrophages to determine if their products can protect against *Yersinia*-induced cell death. The experiments proposed in this R21 application will augment specific aim 3 of the parent R01 grant (AI43389-03 "Modulation of Host Signaling Functions by *Yersinia* Yops"). Specific aim 3 is to elucidate the mechanism of YopJ-induced apoptosis by identifying functional interactions between YopJ and components of host signaling pathways. The proposed experiments are consistent with the exploratory/developmental nature of the R21 application because they will employ established genomic approaches to characterize genome-wide transcriptional responses and to facilitate gene discovery.

**Grant:** 1R21AI053778-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** SCHOOLNIK, GARY K MD  
**Title:** Microarray Studies of Vibrio cholerae EPS Regulation  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): The principal reservoirs of *Vibrio cholerae* O1 in nature are rivers, estuaries and marine habitats. The El Tor biotype of *V. cholerae* O1 exhibits phase variation between the smooth and rugose morphotypes. Of these, the rugose variant is thought to be particularly well adapted for long-term survival in natural water sources: it produces an extracellular polysaccharide (EPS) that enables it to form three-dimensional biofilms and it confers resistance to chlorine and hydrogen peroxide. EPS expression is positively regulated by VpsR, a response regulator of the two-component signal transduction regulator class and negatively regulated by HapR, a homologue of the LuxR regulator of *V. harveyi*. The main purpose of this proposal is to capitalize on the recent publication of the *V. cholerae* O1 El Tor genome sequence by using microarray expression profiling and bioinformatic tools to capture and analyze the transcriptomes of the smooth and rugose colonial morphotypes and to define the VpsR and HapR regulons. This will greatly facilitate the goals of the parent grant (AI43422, "EPS: structure, regulation and function") and in particular Specific Aim #2 of that grant which focuses on the regulation of EPS expression. Using a whole-genome DNA *V. cholerae* microarray fabricated in the laboratory of the P.I., expression profiles will be obtained from the smooth and rugose colony types and from hapR and vpsR mutants. Clustering algorithms will be used to identify genes that are differentially regulated in each colony type and genes whose regulation is HapR or VpsR-dependent. A novel bioinformatic tool, BioProspector, will be employed to identify HapR or VpsR consensus sequences in the upstream promoter regions of the genes believed to compose the HapR and VpsR regulons. Gel retardation assays will be performed to corroborate the BioProspector-derived predictions. The possible significance of this project comes from the likelihood that it will provide a deeper understanding of the nature of "rugosity" and thus of the capacity of *V. cholerae* O1 El Tor to persist in environmental reservoirs. This proposal is consistent with the exploratory/development nature of the R21 mechanism because it explores the combined use of microarray expression profiling and bioinformatics to define regulons--potentially a very powerful, but incompletely examined application of this methodology.

**Grant:** 1R21AI053796-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** LEE, RICHARD E PHD CHEMISTRY  
**Title:** Development of TB cell wall biogenesis inhibitors  
**Institution:** UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN  
CTR  
**Project Period:** 2002/09/29-2004/08/31

DESCRIPTION (provided by applicant): The goal of this proposal is to apply innovative technologies to advance novel sets of inhibitors against the rhamnose and galactose cell wall biosynthesis enzymes of *M. tuberculosis*. Three groups of inhibitor "hits" discovered in the parent grant will be developed into non-toxic, lead compounds that inhibit both their target enzymes and bacterial growth at low concentrations. Hit compounds will progress through a technologically advanced drug development program that includes: (1) structure and diversity guided compound library design; (2) X-ray crystal structure analysis of inhibitor/enzyme complexes; (3) high-throughput parallel combinatorial synthesis. This response to PAS-02-031 is designed to capitalize on the advancements made under the parent grant, AI-33706, in which four enzymes, Rml B-D and Glf, have been shown to be essential for *M. tuberculosis* growth. Also under the parent grant, inhibition assays for each enzyme were developed, "drug-like" structurally diverse libraries were screened (using additional support from AI-46393), and three classes of active compounds were identified. In this proposal, a structure guided drug development cycle will be implemented to optimize each class of active compounds. Initially, computer modeling and the known X-ray structures of the four enzymes will be used to determine the interactions between identified inhibitors and the active-site ligands of their target enzyme. Co-crystal structures of selected hits will be determined by X-ray crystallography and subsequent in silico libraries will be designed based on each of the three scaffolds. The libraries will be filtered for compounds that have low toxicity, good pharmacokinetics, tight binding to the active site and substantial chemical diversity. These optimized libraries will then be synthesized via high-throughput, parallel synthesis and tested for enzyme inhibition, anti-mycobacterial activity against *M. tuberculosis*, and selective toxicity in human cell lines. This cycle will then be repeated until highly active, non-toxic leads are produced. The innovative drug development program in this proposal will complement research on the parent grant and significantly progress this research forward, towards the ultimate goal of developing more effective drugs for the treatment of tuberculosis.

**Grant:** 1R21AI053809-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** SILVERMAN, NEAL S BA  
**Title:** Genetic and molecular analysis of Yersinia YopJ  
**Institution:** UNIV OF MASSACHUSETTS MED SCH WORCHESTER, MA  
WORCESTER  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Yersinia pestis is the causative agent of the plague and an important bioterrorism threat. Pneumonic plague is readily transmitted from person to person. Thus, a terrorist attack with Yersinia pestis has the potential to rapidly spread and overwhelm any available medical services. Yersinia Yop proteins are key virulence factors that are translocated into the cytoplasm of host cells, where they potently inhibit the immune response enabling Yersinia to grow almost unimpeded in vivo. YopJ inhibits critical signaling pathways used by the host to rapidly activate the immune response, including the NF-kappaB and mitogen activated protein kinase (MAPK) signaling pathways. YopJ is thought to be an ubiquitin-like protein protease. It has been proposed that YopJ functions by removing SUMO from an unidentified target protein(s). However, the substrate for YopJ protease activity remains mysterious and the molecular mechanisms by which YopJ inhibits signaling are still unclear. We propose two independent approaches to establish the mechanism(s) used by YopJ to block NF-kappaB and MAPK activation. TRAF6 is a critical component of the signaling pathway which activates both the NF-kappaB and MAPK signaling cascades in response to infection and is an obvious candidate target for YopJ. In the first Aim, the possibility that YopJ deubiquitinates, and thus deactivates TRAF6 will be tested in cell culture experiments and by direct in vitro biochemical assays. Humans and insects, including Drosophila melanogaster, use highly conserved signaling pathways to activate their immune response, including NF-kappaB and MAPKs. YopJ is likely to inhibit the fly immune response. Thus, we propose a second Aim that takes advantage of the enormous power of Drosophila genetics. Using transgenic flies and established genetic techniques, we will analyze the role of the putative deSUMOylating activity of YopJ, and identify the relevant components of the YopJ pathway. By identifying the true targets of YopJ, new therapeutic means to treat the plague can be devised, thus reducing the potential impact of a bioterrorist attack.

**Grant:** 1R21AI053813-01  
**Program Director:** NEAR, KAREN A.  
**Principal Investigator:** REYNOLDS, ROBERT C PHD  
**Title:** Crystallization of the Galactosyltransferase from Mtb  
**Institution:** SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, AL  
**Project Period:** 2002/09/15-2005/08/31

DESCRIPTION (provided by applicant): This application is in response to PAS-02-031 for a supplement to our current NIAID-funded program AI45317 entitled Glycosyltransferases as Drug Targets in Mycobacteria. This supplemental program will involve the expression, purification, and crystallization of the galactosyltransferase (Rv3808c) from *Mycobacterium tuberculosis*. The galactosyltransferase adds galactofuranose units to the growing galactan, a polysaccharide that anchors the mycolylarabinan superstructure and is critical to mycobacterial cell wall integrity in the harsh environment of the human macrophage. This effort is responsive to the announcement in that it supplements our current program by applying modern structural biology techniques to obtain a structure of a protein targeted for drug development under our current grant. This protein was not available at the time of our application, and the information provided through the application of the requested supplemental funds will give us a specific picture of the protein active site allowing more rapid development of drugs to target this critical mycobacterial enzyme. As well, this grant is responsive to the PA in that it addresses a new drug target in tuberculosis, and may have implications in the treatment of multi-drug resistant tuberculosis (MDR-TB), a critical health problem worldwide. Tuberculosis and its drug resistant forms are the focus of two active NIAID Program Announcements (PA-99-124 & PA-01-113) and is considered a potential biological weapon by the Department of Defense. This application involves modern structural biology that may not be considered as innovative as recent techniques in bioinformatics, genomics, and microarray technology. This effort, however, is innovative in the choice of target and the impact that structural biology can have on our funded program.

**Grant:** 2R37AI026756-15  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** RADOLF, JUSTIN D MD CLINICAL MEDICAL SCIENCES, OTHER  
**Title:** T.pallidum Membrane Immunogens and Anti-Oxidant Defense  
**Institution:** UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT  
MED/DNT  
**Project Period:** 1992/07/01-2007/06/30

Although much has been learned about venereal syphilis in the nearly one hundred years since the discovery of its etiologic agent, *Treponema pallidum* subsp. *pallidum*, the disease remains highly enigmatic. Our limited understanding of this chronic, systemic, sexually transmitted infection reflects the many peculiarities of the syphilis spirochete, which include the fragility and unusual molecular architecture of its outer membrane, an extremely narrow mammalian host range, and its inability to replicate continuously in artificial medium. A central theme of our research program has been and will continue to be the identification and characterization of rare outer membrane proteins. There are two principal reasons why we believe that this arduous search is about to reach a successful conclusion. First, using a lipophilic photoactivatable probe, we have identified what appears to be an authentic rare outer membrane protein, designate p30.5. Second, we now have at our disposal the *T. pallidum* genomic sequence, an extremely powerful tool which now enables us to survey in silico the entire treponemal chromosome for additional candidate outer membrane proteins. Equally important, the *T. pallidum* genomic sequence has provided an invaluable platform for our investigations of physiological processes (i.e., trace metal acquisition and resistance to oxidative stress) which are fundamental to host-pathogen relations during syphilitic infection. Though clearly departures from our traditional areas of concentration, these new avenues of investigation are, nonetheless, outgrowths of our longstanding commitment to delineating in molecular terms the interface between the spirochete and its obligate human host. During the current funding interval, we have made substantial progress towards our long term objective of relating *T. pallidum* membrane biology to syphilis pathogenesis. In the present proposal, we hope to extend this process by integrating molecular, ultrastructural, and physiological information into a more complete picture of how the parasite sustains itself within the hostile host milieu. To achieve this end, we will molecularly characterize *T. pallidum* rare outer membrane proteins (Specific Aim One); define the roles of the Tro and TP0034 transporters in trace metal acquisition by *T. pallidum* (Specific Aim Two); and characterize key components of *T. pallidum*'s defense against oxidative stress (Specific Aim Three).

**Grant:** 1U01AI053858-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** PETERSON, JOHNNY W PHD  
MICROBIOLOGY:MICROBIO  
OGY-UNSPEC  
**Title:** Development of Therapeutic Inhibitors to Anthrax Toxins  
**Institution:** UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX  
GALVESTON  
**Project Period:** 2002/09/30-2007/07/31

DESCRIPTION (provided by applicant): Anthrax is a highly infectious disease caused by *Bacillus anthracis*, and aerosolization of the dried bacterial spores is a major biological warfare and bioterrorism threat. Two plasmid-encoded anthrax toxins are essential for bacterial virulence. Edema toxin (EdTx) is comprised of edema factor (EF) and protective antigen (PA), while lethal toxin (LeTx) is a molecular complex of lethal factor (LF) and PA. EF is a secreted calmodulin-dependent adenylyl cyclase enzyme that causes tissue edema, and LF is a uniquely selective  $Zn^{++}$ -metalloprotease that inactivates important cell-signaling enzymes (mitogen-activated protein kinase kinases [MAPKKs]) in mammalian cells. PA is the receptor-binding component, which delivers the catalytic components into the cytosol of cells. Our hypothesis is that novel drugs (specific inhibitors of anthrax toxins) can be prepared to reduce the virulence of these bacteria for humans/animals and provide a new therapeutic adjunct to antibiotic therapy and vaccination. The proposal is based on our extensive preliminary studies of new heterocyclic compounds (e.g., prostaglandin E2-L-histidine) that specifically block the adenylyl cyclase activity of EF, and knowledge of metalloprotease inhibitors that block LF activity. Objective 1 will evaluate the capacity of PGE2-L-histidine and PGE2-imidazole to reduce adenylyl cyclase activity of the EF toxin component using an in vitro enzyme assay. We will then use these data to design other inhibitors and dock them on the known crystal structures of EF and other adenylyl cyclases. Objective 2 will identify and characterize inhibitors that block the  $Zn^{++}$ -metalloprotease activity of LF, and we will use these data in 3D-Quantitative structure activity relationship (QSAR) computations to optimize the enzyme inhibitors. Objective 3 will test the effectiveness of the EF and LF inhibitors in protecting cultured cells and mice challenged with toxins or *B. anthracis*. Objective 4 will evaluate the pharmacologic and toxicologic properties of these toxin inhibitors in experimental animals and establish their relative safety. Development of new drugs for anthrax by combining the inhibitors of EF and LF should reduce the virulence of *B. anthracis*, increase the efficacy of antibiotics, promote killing of the bacteria by phagocytes, and enhance vaccine-induced immunity.

**Grant:** 1U01AI053860-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** KOKAI-KUN, JOHN F PHD  
**Title:** Nisin-based topical formulation for treatment of anthrax  
**Institution:** BIOSYNEXUS, INC. GAITHERSBURG, MD  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): We propose to develop and produce a formulation, based on the antimicrobial peptide nisin, that is safe for use on human skin and rapidly bactericidal for vegetative cells and spores of the microbial pathogen *Bacillus anthracis*, the agent responsible for anthrax. The final product is intended for topical use as a decontaminant and disinfectant by military personnel, civilian victims, first-responders, and medical personnel exposed to *B. anthracis* in a biowarfare or bioterrorism attack. The project will build on the existing knowledge and technology surrounding nisin and its applications in the food industry to control food-spoilage organisms, including the outgrowth of spores of *Bacillus* spp. and *Clostridium* spp. and in the veterinary field as a topical sanitizer targeting Gram-positive and Gram-negative pathogens. Formulation development will combine nisin with chelators, non-ionic surfactants, and essential oils that have been shown to enhance nisin activity against Gram-negative and Gram-positive bacteria, including spores. Prototype formulations tested will include lotions, moist wipes, and creams. Testing will be conducted in the presence and absence of serum, using in vitro assays and in vivo animal topical infection models. The final product is intended to minimize the danger for those exposed to *B. anthracis* and should also be effective for disinfection of other bioterrorism agents and for nosocomial pathogens in the healthcare setting.



**Grant:** 2R01AR002255-38A1  
**Program Director:** GRETZ, ELIZABETH  
**Principal Investigator:** COLE, BARRY C PHD  
**Title:** Mycoplasma Superantigen & RA MHC Susceptibility Alleles  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 1978/03/01-2007/03/31

DESCRIPTION (provided by applicant): Autoimmune diseases such as rheumatoid arthritis (RA) are currently considered to be due to a complex interplay of host genetics with environmental triggering agents, which include infectious agents bacteria, viruses or mycoplasmas. The overall goals of this project are to determine how microbial products such as superantigens (SAGs) might interact with RA MHC-susceptibility alleles to drive a type 1 inflammatory cytokine profile that might trigger active disease in the human host. As a model we will use the newly developed murine class II knockout mice that display various human MHC molecules that predispose to development of collagen arthritis in mice. Mice expressing these molecules will be tested for their cytokine profiles in response to in vivo exposure to the mycoplasma SAg, MAM and other bacterial SAGs using RT PCR and ELISA methodologies. We shall also investigate the mechanisms of any differences seen including the potential role of MHC binding, cell type, role of co-stimulatory molecules, and region of the SAg molecule responsible. Also we will investigate the pathogenetic effects of M. arthritidis in these "humanized" mice and determine strategies to overcome the effect of the superantigen MAM in initiating disease pathogenesis by modification of the cytokine milieu.

**Grant:** 1R01AR047948-01A1  
**Program Director:** SERRATE-SZTEIN, SUSANA  
**Principal Investigator:** DESILVA, ARAVINDA M PHD  
**Title:** Population dynamics of *Borrelia* transmitted by ticks  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2002/04/15-2007/03/31

**DESCRIPTION:** (provided by the applicant): Previous studies have demonstrated that Lyme disease spirochetes (*Borrelia burgdorferi*) delivered by ticks are resistant to immune serum unlike cultured bacteria, which are unable to infect mice in the presence of immune serum. The hypothesis being tested in this proposal is that events occurring within feeding ticks transform a homogeneous population of spirochetes into an antigenically and genetically heterogeneous population that is better adapted to evading host immunity and establish an infection in the host. Preliminary studies with the recombinogenic *vlsE* locus indicate that spirochetes within feeding ticks contain a greater number of *vlsE* alleles than bacteria within unfed ticks, possibly because tick feeding stimulates recombination at specific loci. The goal of specific aim 1 is to characterize the distribution of *vlsE* alleles in a spirochete population at distinct states (mice, larval ticks, unfed nymphal ticks, feeding nymphal ticks) as the bacteria go through one complete transmission cycle. The goal of specific aim 2 is to study the mechanism responsible for changing the distribution of *vlsE* alleles within ticks. An artificial feeding method will be used to introduce clonal populations of bacteria into ticks and these clonal populations will be followed to determine the primary mechanism responsible for generating new alleles during tick feeding. The goal of specific aim 3 is to determine if the tick gut is a major site of bacterial phase variation. Studies with Outer surface proteins (Osp) A and C indicate that spirochetes in the gut turn on and off different lipoproteins during the blood meal. Experiments will be done to characterize the expression pattern of an additional nine well-characterized *B. burgdorferi* surface proteins to determine the extent of phase variation in the gut. Preliminary data on *ospC* expression support the hypothesis that *OspC* is a transmission factor required for the movement of bacteria from the tick gut to the salivary gland. Under specific aim 4, bacterial mutants missing a complete *ospC* gene will be introduced into ticks to determine if the mutants are capable of crossing the gut epithelium and invading the salivary glands of the tick. Our central hypothesis that tick transmission leads to the introduction of a heterogeneous population of bacteria into the host has important implications for Lyme disease pathogenesis and vaccine development.

**Grant:** 1R01AR048223-01  
**Program Director:** MOSHELL, ALAN N.  
**Principal Investigator:** STANLEY, JOHN R MD CLINICAL MEDICAL SCIENCES, OTHER  
**Title:** Mechanisms of Blister Formation by Staphylococcal Toxins  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 2002/04/01-2007/03/31

Exfoliative toxin A (ETA), produced by *Staphylococcus aureus*, causes staphylococcal scalded skin syndrome (SSSS) and its more localized form, bullous impetigo. The crystal structure of ETA suggests that it is a serine protease with an inactive catalytic site which becomes activated when ETA binds a specific receptor. In pemphigus foliaceus autoantibodies that cause dysfunction of Dsg 1 cause blisters identical to those caused by ETA in the superficial epidermis of mouse and man. Therefore, we hypothesize that Dsg 1 specifically binds and activates ETA, which in turn cleaves the bound Dsg 1, resulting in blister formation. We propose that another staphylococcal toxin, exfoliative toxin B (ETB), that also causes bullous impetigo and SSSS, is also activated by, and cleaves, Dsg 1. Finally, we hypothesize that binding of ETA to Dsg 1 and/or cleavage of Dsg 1 by ETA might elicit an autoimmune response against Dsg 1, thus suggesting a mechanism for autoantibody production in PF patients. We have shown that ETA cleaves Dsg 1. Specific aim 1 will characterize this cleavage by determining if cleavage is dependent on Dsg 1 conformation, and by defining the site of cleavage and the domains of Dsg 1 needed for cleavage. Aim 2 will characterize binding of ETA to Dsg 1, and define the domains of each necessary. Aim 3 will determine, using Dsg 3 knockout and involucrin-Dsg 3 transgenic mice, if compensation by Dsg 3 can compensate for ETA-induced loss of function of Dsg 1, thereby explaining the sites of blister localization. Aim 4 contains studies designed to define the kinetics of Dsg 1 cleavage by ETA. Aim 5 will extend the results of the previous aims to include the mechanisms of action of ETB. The final aim will determine if patients with bullous impetigo and SSSS develop an antibody response against Dsg 1, if patients with pemphigus foliaceus have an enhanced immune response against ETA and ETB, and if mice injected with ETA develop an immune response against Dsg 1. These studies will provide insight regarding the molecular pathophysiology of a very common disease, bullous impetigo, and, for the first time, identify a potential trigger or exacerbating factor in a tissue-specific autoimmune disease, pemphigus.

**Grant:** 1R01AR048842-01  
**Program Director:** PANAGIS, JAMES S.  
**Principal Investigator:** MARRIOTT, IAN PHD  
**Title:** Osteoblast-derived inflammatory mediators in infection  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHARLOTTE, NC  
CHARLOTTE  
**Project Period:** 2002/08/12-2005/07/31

Staphylococcus aureus is the single most common cause of osteomyelitis in humans. Incidences of osteomyelitis caused by *S. aureus* have increased dramatically in recent years, in part, due to the appearance of community-acquired antibiotic resistant strains of this pathogen. The recent identification of *S. aureus* isolates with reduced vancomycin susceptibility indicates the possibility of a pathogen resistant to all current forms of therapy. Thus, the increasing prevalence of *S. aureus* associated bone infections make understanding the pathogenesis of this organism imperative. Recently, we have described the surprising ability of bone-forming osteoblasts to secrete the key inflammatory cytokine, IL-12, when exposed to *S. aureus*. This finding is particularly significant given the central role IL-12 plays in the induction of cell-mediated immune responses. Such immune responses are essential for the successful elimination of intracellular pathogens such as *S. aureus*. The ability of these cells to produce IL-12 points to a previously unrecognized role for osteoblasts in the generation of protective immune responses and the resolution of infection of bone. In this application we will determine whether the production of IL-12 seen in vitro is reproducible in both an in situ-like organ cultures of neonatal mouse calvaria and in vivo using an animal model developed in our laboratory. Furthermore, we will establish the clinical relevancy of these findings using bone sections from patients with *S. aureus* osteomyelitis. Finally, we propose to investigate the mechanisms responsible for inducing the production of this important cytokine by osteoblasts. Specifically, we will determine whether the expression of bacterial recognition Toll- like receptors underlie the ability of bacteria to induce IL-12 production in cultured osteoblasts. These studies are anticipated to demonstrate that bone-forming osteoblasts respond to *S. aureus* by the production of this pivotal inflammatory molecule, thereby expanding the recognized role of these cells to include being key components in host responses during bone infection.

<b>Grant:</b>	1R03AR047640-01A1	
<b>Program Director:</b>	SERRATE-SZTEIN, SUSANA	
<b>Principal Investigator:</b>	PAL, UTPAL	PHD
<b>Title:</b>	OspA-mediated Borrelia adherence to tick gut	
<b>Institution:</b>	YALE UNIVERSITY	NEW HAVEN, CT
<b>Project Period:</b>	2002/04/15-2005/03/31	

DESCRIPTION (provided by applicant): The present proposal aims to understand OspA-mediated adherence of spirochete *Borrelia burgdorferi* during Lyme disease. *B. burgdorferi* cycles between an arthropod vector and a mammalian host in nature and transmitted to humans by the bite of an infected *Ixodes scapularis* tick. Outer surface protein A, a lipoprotein found on the surface of the bacterium, forms the basis of the recombinant human vaccine against Lyme disease. Differential expression of ospA during life cycle of the spirochete indicates the important temporal and tissue-specific function of the protein by *B. burgdorferi* in the tick gut. Our preliminary data now show that OspA mediates *B. Burgdorferi* attachment to tick gut via binding to a tick gut protein. In the present study we will characterize OspA binding to the tick gut, including the identification of OspA epitopes that facilitate this interaction. Then we will identify and clone the tick receptor for OspA. Since OspA is also expressed sometimes during late stages of arthritis, we will also seek to test whether OspA mediates *B. Burgdorferi* adherence in the joints. These studies should provide new knowledge that may be useful for developing an alternate OspA or OspA-receptor based Lyme disease vaccine and also to understand pathogenesis of Lyme disease.

**Grant:** 1R03AR048973-01  
**Program Director:** GRETZ, ELIZABETH  
**Principal Investigator:** ARULANANDAM, BERNARD P PHD  
**Title:** The Role of IgA in S. aureus Mediated Inflammation  
**Institution:** UNIVERSITY OF TEXAS SAN ANTONIO SAN ANTONIO, TX  
**Project Period:** 2002/09/01-2005/08/31

DESCRIPTION (provided by applicant): Staphylococcus aureus infections often lead to hematogenous spread that result in inflammation and joint destruction. Although the virulence factors associated with this bacteria have been defined, the role of the host immune response in limiting the inflammatory process is still poorly understood. Using mice with a targeted disruption in IgA gene expression (IgA<sup>-/-</sup> mice), we have recently shown another yet unrecognized role for IgA. Specifically, IgA appears to serve an essential role in maintenance of overt inflammatory cytokine and nitric oxide (NO) production. We will now investigate in detail the basis for the immunoregulatory role of IgA in modulating systemic inflammatory responses upon bacterial insult. Using a strain of S. aureus that induces sepsis and joint destruction, we will initially examine the precise role of IgA in limiting inflammatory processes using IgA<sup>-/-</sup> mice. Bacteremia will be assessed in the blood, spleen and kidneys after infection. Inflammatory cytokine production will be monitored in the blood and lymphoid tissues by ELISA and ribonuclease protection analysis respectively. Inflammation in the joints of these animals will be assessed by immunohistochemistry. Since NO production may have both detrimental and beneficial effects during S. aureus infection, we will examine if the absence of IgA potentiates NO expression in the joints of infected mice by histological analyses. Given that the NF- $\kappa$ B pathway is essential in the regulation of iNOS and NO production, we will examine how the absence of IgA affects various components of this pathway by cellular and molecular analyses. Finally we will determine if IgA regulates an inhibitory signaling pathway in macrophages upon bacterial stimulation. Together, these studies will determine if IgA modulates inflammatory responses to systemic bacterial infection by direct interaction with the innate immune system. The results will provide insight on the role of serum IgA to regulate inflammatory processes such as bacterial sepsis and may lead to the use of use of IgA as a therapeutic anti-inflammatory agent.

**Grant:** 2P01CA012582-31  
**Program Director:** WU, ROY S  
**Principal Investigator:** MORTON, DONALD L MD OTHER CL  
MED:CLINICAL  
MEDICINE,UNSPEC  
**Title:** SURGERY, IMMUNOLOGY AND IMMUNOTHERAPY OF HUMAN CANCER  
**Institution:** JOHN WAYNE CANCER INSTITUTE SANTA MONICA, CA  
**Project Period:** 1977/07/01-2007/03/31

DESCRIPTION (provided by applicant) The overall goal of this Program is to improve the survival of cancer patients by active specific immunotherapy. To achieve this goal, we will utilize melanoma as a model neoplasm. This renewal application represents a continuation of our previous studies with polyvalent melanoma cell vaccine (PMCV) and investigates the in vivo and in vitro mechanisms of PMCV anticancer activity. The first project is a new project for this renewal which will build on the past successes of this program by utilizing both adoptive and active immunotherapy and targeted vaccine designs to induce antigen specific immune responses. Patients' dendritic cells will be fused with the autologous tumor cells present in the PMCV by electrofusion. Phase I/II clinical trials will be conducted. The second project will continue to utilize already established, as well as developing new, molecular assays using both RNA and DNA markers present in blood and tumors as surrogates to predict disease progression, outcome and treatment efficacy. The third project will complete a randomized Phase III trial in AJCC stage III melanoma to compare PMCV plus BCG compared to BCG alone measuring disease free and overall survival after surgery. This project will also develop an immunological response model for predicting outcomes following vaccine therapy, undertake studies utilizing cytokines to improve vaccine immunotherapy and develop new tumor markers to detect subclinical metastatic disease. All projects rely upon the support of four cores: Administrative and Clinical Support Services, Biostatistics, Serum, Lymphocyte and Tissue Collection, and Molecular Support.

**Grant:** 2R01CA076461-05A1  
**Program Director:** HALLOCK, YALI  
**Principal Investigator:** FLOSS, HEINZ G PHD CHEMISTRY:ORGANIC  
**Title:** Biosynthesis of Maytansinoids and Analogs  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1998/01/15-2006/06/30

DESCRIPTION (provided by applicant): The ansamitocin family of ansamycin-type microbial metabolites produced by the Actinomycete *Actinosynnema pretiosum* and the structurally almost identical plant-derived maytansine and its congeners are extraordinarily potent antitumor agents. Despite its toxicity, maytansine at one time was considered an outstanding candidate for clinical development, but it failed to show significant efficacy in phase II clinical trials, probably due to dose-limiting toxicity. However, interest in these compounds continues, e.g., as "warheads" for antibody-targeted delivery, and their high potency calls for further efforts to modify their structures with the goal of identifying analogs which retain high antitumor activity coupled with lower toxicity than the parent compounds. The structural complexity of these compounds limits chemical approaches to modified structures to those accessible by semi-synthesis from the natural product starting materials; more deep-seated backbone structural modifications require biochemical approaches based on genetic alteration of the biosynthetic machinery generating the parent compounds. With this rationale in mind we have cloned and sequenced the ansamitocin (asm) biosynthetic gene cluster from *A. pretiosum* and are in the process of analyzing the functions of its individual genes. In the next 4-year period of this grant we wish to continue this work by determining the functions of all the downstream modification genes/enzymes, expressing the polyketide synthase (PKS) assembling the backbone of ansamitocin in a heterologous host and studying its structure and mode of operation, and clarifying the structure and mode of formation of a rare polyketide chain extension unit required for the function of the asm PKS. Based on the insights gained from this work, we will then assemble a system, which allows the expression of all the asm biosynthetic genes to produce ansamitocins in a heterologous host, *Streptomyces coelicolor*, from a series of gene cassettes under the control of an external promoter. Once this system is established, we will demonstrate the feasibility of producing ansamitocin analogs by introducing genetic modifications into the expressed gene cluster. In addition we plan to investigate the genetic control of ansamitocin production in *A. pretiosum* with the aim to increase yields of the parent and engineered mutant compounds. This work will thus provide the tools for the preparation of structural analogs of the ansamitocins, which can be evaluated for an improved therapeutic ratio as anticancer agents.



**Grant:** 2R01CA077248-05  
**Program Director:** HALLOCK, YALI  
**Principal Investigator:** KHOSLA, CHAITAN S  
**Title:** Bacterial Aromatic Polyketide Synthases  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 1998/08/20-2007/03/31

**DESCRIPTION:** (provided by applicant) Polyketide synthases (PKSs) are a family of multi-enzyme assemblies that catalyze the biosynthesis of numerous structurally complex and biologically important natural products. Bacterial aromatic PKSs are one subclass of PKSs responsible for the biosynthesis of natural products such as doxorubicin and tetracycline. They are composed of 3-10 distinct subunits, which together synthesize a polyfunctional aromatic product. The modularity of these multi-enzyme systems has been exploited via genetic engineering for the biosynthesis of numerous "unnatural" natural products. However, our understanding of the structural and mechanistic principles by which these remarkable enzymes assemble and catalyze multi-step transformations involving highly reactive intermediates is rudimentary. During the past proposal period we have expressed, purified, and reconstituted the actinorhodin (act), tetracenomycin (tcm), and parts of the R1128 PKSs. This has allowed us to probe the properties of selected aromatic PKS components using a combination of mutagenesis, protein chemical, structural (NMR and X-ray crystallography), and biosynthetic engineering approaches. The specific goals for the next proposal period are: 1) Development of improved expression systems, purification procedures, and assay systems for aromatic PKSs and their components, 2) Further structural and mechanistic analysis, and biosynthetic exploitation of the unusual primer unit tolerance of the R1128 PKS, 3) Further structural and mechanistic analysis, and engineering of the chain length specificity of the act, tcm, and possibly other minimal PKSs, 4) Dissecting and engineering the malonyl-CoA selectivity of the malonyl-CoA:acyl carrier protein acyltransferase, 5) Analyzing the kinetic consequences of protein-protein interactions between the ketosynthase, acyl carrier protein, and acyltransferase, and 6) Engineering a hybrid PKS that contains components of an aromatic and a modular PKS. These studies, which follow logically from our results thus far, are expected to provide interesting and important insights into structure-function relationships within this remarkable family of multi-enzyme assemblies. Furthermore, knowledge acquired in the process could expand the utility of bacterial aromatic PKSs (and possibly other PKSs as well) for the engineered biosynthesis of novel natural products.

**Grant:** 1R01CA090860-01A1  
**Program Director:** PELROY, RICHARD  
**Principal Investigator:** KOW, YOKE W PHD PHOTOBIOLOGY  
**Title:** Repair of Clustered DNA Damages  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 2002/01/11-2005/12/31

DESCRIPTION (provided by applicant): Ionizing radiation produces a wide spectrum of DNA damages including base and sugar damages, single and double strand breaks, abasic sites, DNA-protein and DNA-DNA crosslinks as well as multiply damage sites(MIDS). Double strand breaks are repaired either by homologous recombination or non-homologous end-joining mechanism. Despite the fact that double strand break is a lethal lesion, lethality induced by ionizing radiation cannot be fully explained by the amount of double strand breaks formed. It was suggested by Ward that a significant amount of cell killing by low LET radiation at biological relevant doses is due to the production of MDS, a cluster of damages within a localized region. There is increasing evidence that MDS are biologically important and might contribute significantly to lethality and mutagenesis induced by ionizing radiation. The long-term goal for this project is to understand the biological consequences of MDS. Two approaches are taken in order to achieve this goal. Aims 1 to 2 are directed to elucidate the in vivo biological consequences of MDS. A yeast shuttle plasmid, pRS413, containing various MDS will be constructed and used to transform E. coli of various repair backgrounds. The lethality conferred by various MDS will be scored by measuring the survival of damaged pRS413 and the mutagenicity of MDS by direct sequencing of the mutant progeny plasmid obtained after transformation. In addition, in vitro processing of these MDS will be studied in Aims 3 and 4. Aim 3 is directed to examine the in vitro processing of DNA containing tandem lesions by E. coli BER enzymes including endonucleases III and VIII, formamidopyrimidine N-glycosylase and 5' AP endonucleases from E.coli. The nature of the reaction products and the kinetics of removal for each of the lesions within the MDS will be elucidated. In addition, a complete in vitro analysis of the possible enzymatic steps involved in the repair of tandem lesions will also be performed. Aim 4 is directed to examine whether E. coli DNA binding protein, such as HU protein can mediate the sequential repair of closely opposed lesion. It is believed that a comprehensive study involving in vivo biological and in vitro enzymatic studies will provide significant insight into understanding the biological consequences of MDS, and thus the genotoxic and mutagenic effect of ionizing radiation.

<b>Grant:</b>	1R01CA093457-01	
<b>Program Director:</b>	DUBOIS, RONALD J.	
<b>Principal Investigator:</b>	KOZMIN, SERGEY A	PHD
<b>Title:</b>	The Synthesis of Leucascandrolide A	
<b>Institution:</b>	UNIVERSITY OF CHICAGO	CHICAGO, IL
<b>Project Period:</b>	2002/01/04-2005/12/31	

The primary objective of this research program is to develop a practical synthesis of leucascandrolide A (1), a novel marine macrolide isolated by Pietra in 1996. In preliminary in vitro studies, leucascandrolide A displayed a potent cytotoxicity, and strong inhibition of the pathogenic yeast *Candida albicans*. Due to the difficulty of isolation of leucascandrolide A, combined with the presently unknown biogenetic origin, an efficient chemical synthesis represents the only viable approach to this rare natural product. Having completed the synthesis of the C(1)-C(15) fragment of this natural product, we propose a convergent, fully stereocontrolled synthetic approach to leucascandrolide A, suitable for the production of sufficient amount of this natural product for a comprehensive biological evaluation. In addition, we will develop the new synthetic methods including asymmetric Prins desymmetrization, tandem and catalytic asymmetric hydrosilylations, designed to provide an access to a variety of valuable synthetic intermediates. Starting at the level of basic research in organic and organometallic synthesis, it is our ultimate objective to provide new directions for the development of new anticancer therapeutic agents.

**Grant:** 1R01CA095946-01A1  
**Program Director:** ERICKSON, BURDETTE (BUD) W  
**Principal Investigator:** PARSONNET, JULIE  
**Title:** GIS for Extant Data: Modeling H.Pylori and GI Tumors  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 2002/09/30-2005/08/31

DESCRIPTION (provided by applicant): Many large demographic and health datasets exist in the public domain and significant federal resources have been committed to their collection and maintenance. We postulate that, using geographic information systems (GIS) technology, the enormous body of information within these unrelated datasets can be integrated to efficiently explore novel hypotheses. For such purposes, however, precise methods of using GIS have not been well standardized. In this proposal, we intend to develop a method for integrating diverse data sets using GIS and then use the spatial capacities of GIS to answer epidemiologic questions. We will validate these methods using the model of Helicobacter pylori and malignancy. H. pylori is a known cause of stomach cancer, and has been purported to cause colorectal and pancreatic adenocarcinomas and to protect against esophageal adenocarcinoma. The vast array of epidemiologic knowledge on this bacterium and its associated cancers makes it an excellent subject for validation of these methods. We will use GIS to combine data from the U.S. Census, NHANES III, and the SEER cancer registry. We will then assess the spatial correlations between H. pylori infection and specific cancer incidences and mortality rates. Development and validation of this methodology will highlight the utility of GIS in epidemiologic research. It will provide a cost-effective means to harness the power and efficiency of large-scale surveys to address specific hypotheses at low expense, even if they were not considered during the design of the surveys. Application of these methods could potentially allow investigators to use existing data sources to address novel hypotheses that may have otherwise been not feasible to pursue.

**Grant:** 1R01CA097946-01  
**Program Director:** DASCHNER, PHILLIP J  
**Principal Investigator:** PEI, ZHIHENG MD  
**Title:** Bacterial flora in normal esophagus & reflux disorders  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY  
**Project Period:** 2002/06/01-2006/05/31

DESCRIPTION (provided by applicant): Gastroesophageal reflux (GERD) is a chronic inflammatory disease affecting millions of Americans leading to the development of gastric/intestinal metaplasia (Barrett's esophagus), which is a precursor for adenocarcinoma (Ca). Although data from the stomach and colon suggest that colonizing bacteria are essential in inflammation-induced cancer development, little is known about esophageal bacterial flora. The long-term goal of this project is to define the role of bacteria in the progression of GERD to Barrett's esophagus and Ca. Our hypothesis is that bacterial flora exist in the normal esophagus, changing in the evolution of GERD into Barrett's esophagus and Ca. The specific aims are to: 1) define at a population level the bacterial flora in the normal esophagus and the esophagus with GERD-related diseases, 2) define at a species level the bacterial flora in the normal esophagus and GERD-related diseases, 3) determine host humoral immune responses to esophageal bacteria in patients with GERD-related diseases and controls. To define bacterial flora at a population level. The total number of bacteria per biopsy will be determined by quantitative real-time PCR using universal bacterial 16S rDNA primers with DNA from each biopsy. Specimens from disease groups will be compared for bacterial density (square mm mucosa). To define the flora at a species level, biopsies will be analyzed using sequence-based universal bacterial 16S PCR and cultivation. PCR products will be cloned and sequenced, and species identification accomplished by comparing the sequences with known bacterial 16S sequences. Biopsies also will be cultured in anaerobic, aerobic, and microaerobic conditions, and colonies biochemically defined to a species level. Each disease group will be compared for the species identified and their prevalence. Cultivable whole cell bacterial antigens will be used in ELISA to determine whether the hosts recognize their presence. Serum antibodies also will be examined using immunoblots to identify disease-specific antigens. Significance: (i) Bacterial flora in the GI tract play important roles in pathologic conditions including inflammation and neoplasia. The esophagus is the only part of the GI tract where little is known about the bacterial flora, and the proposed study will assess the existence and complexity of the flora. (ii) While GERD initially results from chemical damage, bacterial overgrowth may promote intestinal metaplasia of the esophagus. The proposed study allows qualitative and quantitative examination of this hypothesis.

**Grant:** 1R03CA094741-01  
**Program Director:** STARKS, VAURICE  
**Principal Investigator:** MADELEINE, MARGARET M PHD  
**Title:** Chlamydia trachomatis and Cervical Cancer  
**Institution:** FRED HUTCHINSON CANCER RESEARCH SEATTLE, WA  
CENTER  
**Project Period:** 2002/03/20-2004/02/29

DESCRIPTION (provided by applicant): Human papillomavirus (HPV) causes common, but usually transient, infections of the cervix that sometimes become cervical cancer. Since few of the women infected with HPV get cervical cancer, we are interested in cofactors in addition to HPV that promote tumor formation. One such possible cofactor is Chlamydia trachomatis, a prevalent, sexually transmitted disease that can infect the cervix for long periods of time. A recent article suggested that specific serotypes of C. trachomatis were associated with the development of cervical squamous cell cancer. We have a population based sample of 500 cervical cancer cases and 300 controls blood samples that have been tested for antibodies to HPV and have been interviewed for risk factors for cervical cancer. The tumor tissue has been tested for HPV DNA by polymerase chain reaction. This resource will allow us to quickly and efficiently test for C. trachomatis in order to evaluate two hypotheses. First, whether an increased risk of cervical carcinoma is associated with C. trachomatis, with an emphasis on the relative risks associated with the three genital C. trachomatis serotype classes. Second, to examine this association separately among women with the two main histologic types of cervical cancer, squamous cell carcinoma (n=300) and adenocarcinoma (n=200) of the cervix. Control subjects selected for this study have serum antibodies to HPV-16 or -18. The benefit of screening and treatment for chlamydial infections might extend to include prevention of the proportion of cervical cancer promoted by infection with specific serotypes of C. trachomatis. If we are able to confirm the relationship between C. trachomatis and cervical cancer, repeated targeted screening of young women for C. trachomatis may become a higher public health priority. Furthermore, our results would help determine whether further follow up of the C. trachomatis and cervical cancer association is warranted in a more expensive, prospective setting.

**Grant:** 1R03CA097475-01  
**Program Director:** WAGNER, PAUL D.  
**Principal Investigator:** LAMPE, JOHANNA W BOTH  
**Title:** Hormone Status Postmenopause: Colonic Bacterial Effects  
**Institution:** FRED HUTCHINSON CANCER RESEARCH SEATTLE, WA  
CENTER  
**Project Period:** 2002/07/02-2004/06/30

DESCRIPTION (provided by applicant) Greater exposure to estrogen throughout a woman's lifetime increases her risk of developing breast cancer. In the gut, microflora play a significant role in the metabolism of estrogens. Therefore, inter-individual differences in host bacterial populations may be a determinant of estrogen exposure and ultimately of breast cancer risk. Colonic microfloral production of equol from the soy isoflavone daidzein serves as a biomarker of a unique intestinal bacterial population. Evidence from several studies suggests that, irrespective of soy intake, women with the capacity to produce equol have hormonal profiles associated with a lower risk of breast cancer. Only about one third of individuals have the yet-to-be-identified bacteria capable of producing equol, and equol-producer status can be determined readily from a urine sample collected after a 3-day soy challenge. To date, there has been no systematic study of the effect of equol producer phenotype on estrogen-dependent biomarkers. We propose to examine the association between equol producer phenotype and current and lifetime measures of reproductive hormone exposure. Circulating reproductive hormones and urinary estrogen metabolites will be examined by equol producer phenotype to evaluate current hormonal exposure. Breast and bone densities (markers of lifetime estrogen exposure) will be examined by equol producer phenotype. We will recruit healthy female volunteers, aged 50 to 75 years, from among the 173 women who participated in the Physical Activity and Total Health Study (NCI R01 CA69334; Effect of Exercise on Sex Hormones in Postmenopausal Women). Women in this study had a baseline mammogram to determine percent breast density by computer-assisted technology, total bone density measured by DEXA, a blood sample collected for the analysis of circulating reproductive hormones, and a urine sample collected for the analysis of estrogen metabolites. Thus, by using this study population, we make efficient use of a comprehensive compilation of existing breast density, bone density, and circulating and urinary hormone data. Each woman will complete a 3-day soy challenge and collect the first-void urine on the fourth day for analysis of equol. We will classify women as equol producers or non-producers and will examine differences in percent breast density by equol producer phenotype. In addition, we will establish whether equol production predicts differences in the measures of hormonal exposure, independently of other factors known to be associated with these measures. The results of this study will provide novel data regarding the relationship of the equol-producer phenotype, a marker of colonic microfloral environment, to lifetime estrogen exposure.

<b>Grant:</b>	1R21CA097945-01	
<b>Program Director:</b>	DASCHNER, PHILLIP J	
<b>Principal Investigator:</b>	KELLEY, SHANA O	PHD
<b>Title:</b>	Detection of H. pylori using electrical DNA sensing	
<b>Institution:</b>	BOSTON COLLEGE	CHESTNUT HILL, MA
<b>Project Period:</b>	2002/04/01-2004/03/31	

DESCRIPTION (provided by applicant): This proposal describes the development of a new class of biosensors exploiting an intrinsic property of DNA: electrical conduction. The capability of dsDNA to conduct electricity, in combination with the ability to measure this conduction in short (<100bp) and specific DNA sequences, will be harnessed to produce sensors with unprecedented sensitivity and portability. Conventional methods of detecting sequences on DNA arrays employ optical detection, and lack the sensitivity required to detect microbial species in clinical samples without extensive processing. The goal of this project is to construct a nanoscale DNA sensor with electrical detection that can sensitively and accurately detect *Helicobacter pylori*, a bacterium associated with gastric ulcers and cancer, in clinical samples. The specific aims of this project include (1) the demonstration of proof-of-principle for electrical DNA sensing carried out with synthetic DNA molecules modeling a unique *H. pylori* gene, and (2) the detection of *H. pylori* in clinical samples using electrical DNA detection.



**Grant:** 1R01DA015018-01A1  
**Program Director:** LAMBERT, ELIZABETH  
**Principal Investigator:** LOWY, FRANKLIN D  
**Title:** Social Networks of *S. aureus* Carriage Among Drug Users  
**Institution:** COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY  
**Project Period:** 2002/09/20-2007/06/30

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is the single most common cause of bacterial infections among drug users and is increasingly recognized as a major cause of life-threatening disease in patients with AIDS. Both drug users and HIV-infected individuals have an increased incidence of colonization and subsequent infection with *S. aureus*. Little is known about the biology of *S. aureus* nasal colonization or the basis for the increased rate of colonization among any of the known high-risk groups. In a community study of active drug users, we found several biologically linked networks of subjects colonized with closely related strains of *S. aureus*. The biologic linkage of the strains, established by pulsed field gel electrophoresis (PFGE), first identified these networks. This study demonstrated a relationship between the drug use setting, a "crack house", and the biologically linked networks of *S. aureus*. The overall goal of this proposal is to define the extent, patterns and mechanisms of *S. aureus* transmission among drug use risk networks. Our specific aims are the following. 1. Establish the baseline distribution of *S. aureus* colonization in drug use networks. 2. Explore the extent to which the prevalence and strain similarity of *S. aureus* colonization are associated with the structure of drug use networks, risk behaviors and features of drug use settings. 3. Examine whether co-factors, such as HIV status influences colonization. 4. Determine the contribution of drug use paraphernalia to the transmission of *S. aureus*. 5. Analyze staphylococcal isolates using PFGE and multilocus sequence typing, in order to define strain identity and the degree of relatedness among isolates, correlate them with international and regional strain databases, supplement social network linkages identified through interview with biologic linkage data and determine which typing tool is most useful in this setting. 6. Use the strain profiles generated by the typing tools to identify virulence determinants that confer an ecologic advantage to these strains, enabling them to colonize, resist antibiotics and spread among subjects. The present study takes advantage of Dr. Miller's preexistent cohort of women drug users and their network members in the Bedford Stuyvesant section of Brooklyn. This will allow for the rapid integration of this investigation into her ongoing study.

**Grant:** 1R01DC005040-01A1  
**Program Director:** PLATT, CHRISTOPHER  
**Principal Investigator:** MINOR, LLOYD B PHD  
OTO/RHINO/LARYNGOLOG  
**Title:** Evaluation of Vestibular Function in Meniere's Disease  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/04/01-2005/03/31

DESCRIPTION:(provided by applicant) The objective of the proposed research is to understand the pathophysiology of the vestibular disturbances in Meniere's disease and how to treat them. The specific effects (both qualitative and quantitative) on vestibular function of both Meniere's disease itself and of intratympanic gentamicin used to alleviate vertigo are unknown and will be determined in the proposed research. The research strategy is to analyze the vestibuloocular reflex (VOR) in three dimensions from responses to stimuli that activate the semicircular canals or the otoliths. Vestibular function will also be evaluated from measurements of the subjective visual vertical and from vestibular-evoked myogenic potentials. The angular VOR evoked by high-frequency, high-acceleration head thrusts will be studied in order to determine the effects of Meniere's disease and of intratympanic gentamicin on the function of individual semicircular canals. The translational VOR in these patients will be evaluated from the responses to rapid, lateral translations of the head. Through comparisons with findings in subjects with normal vestibular function and those with known surgical unilateral vestibular destruction (UVD), these studies will provide a new understanding of the effects of Meniere's disease itself, and the effects of treatment with gentamicin, on individual vestibular end organs. Recovery of the VOR after intratympanic gentamicin and after surgical UVD will be assessed through analyses of the trajectories of eye velocity. The corrective eye movements that reduce the gaze errors that occur as a consequence of diminished vestibular function in the responses to high acceleration angular and translational head movements will also be analyzed. The information derived from this research will have practical import on which vestibular tests are most useful in Meniere's disease, and on deciding when and with what to treat patients with Meniere's disease.

**Grant:** 1R01DC005230-01A1  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** GUAN, XIN-MIN PHD  
**Title:** Molecular Mechanism of Aminoglycoside Ototoxicity  
**Institution:** CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH  
(CINCINNATI)  
**Project Period:** 2002/09/15-2007/08/31

**DESCRIPTION** (provided by applicant): The goal of the proposed research is to elucidate the molecular pathogenetic mechanism of maternally inherited aminoglycoside induced hearing impairment. Aminoglycoside ototoxicity is a major clinical problem. In the United States, almost 4 million courses of aminoglycoside antibiotics are administered annually for infections. It is estimated that between 2 to 5 percent of patients treated with these drugs develop significant hearing loss. In the developing countries, the problem of ototoxic side effects is more acute due to the widespread use of these drugs. Aminoglycoside ototoxicity is a complex multifactorial disorder resulting from interaction between genetic and environmental factors. The aminoglycoside hypersensitivity is often maternally transmitted. Recently, the A1555G mutation in the mitochondrial 12S rRNA gene has been identified as an inherited mutation that predisposes to aminoglycoside ototoxicity. This mutation accounts for a significant portion of patients with aminoglycoside ototoxicity. However, numerous important questions remain unanswered regarding the molecular and biochemical basis for genetic susceptibility to aminoglycoside ototoxicity. We hypothesize that human mitochondrial 12S rRNA, particularly that carrying the A1555G mutation, is the main target of aminoglycosides and that these drugs exert their detrimental effects in the cochlea through an alternation of mitochondrial protein synthesis. We also hypothesize that there are additional mtDNA mutations associated with aminoglycoside ototoxicity. To test these hypotheses, we propose the specific aims as follows: 1) Examination of the binding of these drugs to the decoding site of mitochondrial 12S rRNA. 2). Examination of the mitochondrial specificity and dosage effect on these antibiotics by analysis of growth properties and rate of mitochondrial protein synthesis in A1555G mutation disease cell model. 3) Identification and evaluation of additional mtDNA mutations associated with aminoglycoside ototoxicity by a systemic and extended screening of a large Chinese clinical patient population with aminoglycoside ototoxicity. Success of this project will provide new insight into the pathogenic mechanisms of aminoglycoside-associated ototoxicity, which in turns provide valuable new information and technology for the diagnosis and prevention of this disorder. The data from this study will help to predict which individuals are at risk for ototoxicity, improve the safety of clinical implications for the aminoglycoside-antibiotic therapy, and decrease the incidence of deafness. The ultimate goal of this study is to develop the aminoglycoside analogs with less toxicity and to provide aminoglycoside treatment strategies that prevent irreversible cochlear damage.

**Grant:** 1R01DC005833-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** SMITH, ARNOLD L MD PEDIATRICS:INFECTIO  
DISEASES  
**Title:** Quorum Sensing in H. influenzae otitis  
**Institution:** SEATTLE BIOMEDICAL RESEARCH SEATTLE, WA  
INSTITUTE  
**Project Period:** 2002/09/20-2007/08/31

DESCRIPTION (provided by applicant): Otitis media with effusion (OME) is a significant health problem of children. Haemophilus influenzae is one of the major causes of this disease. Features of H. influenzae OME include frequent recurrences and a failure of eradication with antibiotic administration. One hypothesis explaining these features of H. influenzae OME is that the organism is growing as a biofilm in the middle ear. Bacterial biofilms are characteristically insensitive to antibiotic treatment, as well as incapable of elimination by the host inflammatory response. Evidence of a H. influenzae biofilm in children with OME consists of the presence of short-lived, Haemophilus-specific mRNA in the middle ear fluid of these children. Gram-negative bacterial biofilm formation is dependent upon the synthesis of quorum-sensing transcriptional activators, called autoinducers. We have found that H. influenzae; including those isolated from the middle ear possess a gene (HI0491) capable of synthesizing an autoinducer (AI-2). Insertional inactivation of HI0491 in the H. influenzae laboratory strain Rd KW20 results in a mutant, which lacks the ability to form mature biofilm structures, and has decreased susceptibility to antibiotics, a decreased conjugation frequency and decreased survival in an animal model of OME. In this application, we are seeking to characterize biofilm formation by several prototypic "otitic" H. influenzae in vitro, assess experimental OME caused by these strains in the weanling rat and chinchillas, determine the role of the autoinducer in this disease and define the role of AI-2 in biofilm development in vitro and in vivo. Understanding the role of AI-2 and biofilm formation in OME will permit strategies to prevent or treat this disease to be devised.

**Grant:** 1R01DC005837-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** CAMPAGNARI, ANTHONY A PHD TUMOR IMMUNOLOG  
**Title:** Genetics /Biology of *M. catarrhalis* LOS in Otitis Media  
**Institution:** STATE UNIVERSITY OF NEW YORK AT AMHERST, NY  
BUFFALO  
**Project Period:** 2002/09/20-2007/08/31

DESCRIPTION (provided by applicant): *Moraxella catarrhalis* is a Gram-negative human pathogen, which predominantly causes middle ear infections and sinusitis in infants and children, and lower respiratory tract infections in adults. This organism is the third leading cause of otitis media and it is estimated that approximately 50% of children will become colonized by this bacterium in the first 6 months of life. Recurrent acute otitis media infections have also become prevalent resulting in potential hearing loss and subsequent developmental and learning problems as these children reach school age. Middle ear infections are very common and it is estimated that 80% of children under the age of 3 will experience at least one episode. There are also significant health care costs associated with treatment for otitis media and it has been determined that *M. catarrhalis* is responsible for approximately 3 to 4 million physician office visits annually. This estimate is considered conservative now that over 90% of *M. catarrhalis* clinical isolates produce b-lactamase. Together, these data have stimulated research efforts aimed at identifying specific virulence factors involved in colonization and infection. One prominent bacterial surface component implicated as a potential virulence factor, is the lipooligosaccharide (LOS). Structural studies show that *M. catarrhalis* LOS is similar to the LOS of other Gram-negative human pathogens, including *Neisseria meningitidis*, *Neisseria gonorrhoeae* and *Haemophilus influenzae*. More importantly, these common LOS structures shared by *M. catarrhalis* and these prominent human mucosal pathogens have been implicated as virulence factors. Although *M. catarrhalis* express these LOS epitopes, there have been minimal studies describing the role of LOS in pathogenesis. In addition, there is currently no information available regarding the assembly and expression of this major surface glycolipid. In this proposal we will perform a comprehensive analysis of the genetics and biology of *M. catarrhalis* LOS. These studies will be instrumental to our understanding of the role of LOS in the pathogenesis of otitis media and should provide insight into new strategies designed to prevent this disease. We will test our hypotheses by the following specific aims: (1) Isolate and characterize of the genes involved in the biosynthesis and assembly of *M. catarrhalis* LOS. (2) Perform biologic studies to define the role for *M. catarrhalis* LOS in otitis media.

**Grant:** 1R01DC005840-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** GILSDORF, JANET R MD  
PEDIATRICS:PEDIATRICS-  
UNSPEC  
**Title:** H. influenzae Genes Associated with Acute Otitis Media  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/09/20-2007/08/31

DESCRIPTION (provided by applicant): Acute otitis media results from a complex interplay of host defenses, environmental factors, and virulence characteristics of bacteria. Previous studies suggest that *Haemophilus influenzae* (Hi), an important cause of acute otitis media in children, possess unique genes important in the pathogenesis of this infection. In this project, we will identify potential virulence factors specific for Hi otitis media. Using a subtractive hybridization technique, we will identify DNA fragments present in Hi otitis media isolates and absent in Hi throat isolates. The subtracted DNA fragments will be cloned, sequenced and used to determine their prevalence among large panels of Hi strains grouped by various epidemiologic parameters. Our large collection of Hi strains will be screened for the otitis media associated genes using a gene detection-based microarray consisting of genomes of the Hi strains and associations among these genes as well as between these genes and epidemiologic factors established. The Specific Aims of this project are: 1. Characterize Hi isolated from middle ear effusions of children with acute otitis media and from nasopharyngeal throat specimens of children by biotype, serotype, b-lactamase production and genotype by pulsed field gel electrophoresis. 2. Identify candidate Hi otitis media associated genes through subtractive hybridization. 3. Describe associations among candidate Hi otitis media associated genes and between Hi otitis media associated genes and epidemiologic parameters, including site of isolation, geographic location, persistence of nasopharyngeal colonization and ability to be transmitted child to child, using a microarray of Hi genomic DNA from each strain in our collections. 4. Characterize the Hi otitis media associated genes by size, predicted function, and location in the Hi chromosome. Identification of unique Hi otitis media virulence factors will facilitate the development of novel approaches to the prevention and treatment of Hi otitis media.

**Grant:** 1R01DC005841-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** CHONMAITREE, TASNEE  
**Title:** Pathogenesis of Virus-Induced Acute Otitis Media  
**Institution:** UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX  
GALVESTON  
**Project Period:** 2002/09/20-2007/08/31

DESCRIPTION (provided by applicant): Otitis media (OM), the most common pediatric disease, is recognized to be multifactorial, with complex genetic, environmental and infectious etiologies. Acute otitis media (AOM) usually occurs as a bacterial complication of viral upper respiratory tract infection (URI) in children. Evidence suggests that different types of viruses vary in their ability to induce AOM. The long-term objectives of our research group are to elucidate the contribution of viruses, bacteria and their complex interactions in the pathogenesis of and recovery from AOM, and to identify possible strategies for more effective prevention and/or treatment. The proposed 5-year study will investigate the relationship between "host" and "microbe" in the development of virus-induced AOM. We will explore the pathogenicity of specific respiratory viruses, and the role of proinflammatory cytokines (TNFcx, -IL-1B, IL-6) and their gene regulation in the mechanisms of virus-induced AOM. In Aim 1, we will study differential cytokine expression in virus-induced AOM in vivo and in vitro. We will prospectively follow 210 infants and children, with and without polymorphisms of acute phase cytokine genes (TNFcx(-308, IB-+3953, and IL- 6 alleles) for one year. For 3 weeks after each viral URI episode, we will monitor for the occurrence of AOM. We will compare virus type and cytokine concentrations in respiratory secretions from children who do and who do not develop AOM as a complication. Risk for AOM development will be evaluated for association with cytokine genotypes. In the in vitro experiments, induction of cytokine production by specific viruses will be studied in peripheral blood mononuclear leukocytes from healthy adult volunteers with normal or polymorphic cytokine genes. Aim 2 will retrospectively evaluate the association between polymorphisms of proinflammatory cytokine genes and susceptibility to OM, using peripheral blood from 200 children with and without a history of recurrent OM. These studies will clarify the role of specific respiratory viruses, proinflammatory cytokines, and their gene regulation in the pathogenetic mechanisms of virus-induced AOM. Study results should improve risk identification for recurrent OM in the population, which may facilitate specific prophylactic and therapeutic approaches for the high risk groups. The study will also lay the ground work for the future design of innovative approaches to prevent or reduce morbidity of AOM, such as therapies using specifically targeted viral vaccines, antiviral drugs, and immunomodulators.

**Grant:** 1R01DC005847-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** BAKALETZ, LAUREN O PHD  
**Title:** Antimicrobial Peptides & Innate Immunity in Otitis Media  
**Institution:** CHILDREN'S RESEARCH INSTITUTE COLUMBUS, OH  
**Project Period:** 2002/09/20-2007/08/31

DESCRIPTION (provided by applicant): Antimicrobial polypeptides (APs) are key components of the primary defense mechanisms of mucus membranes, providing first-line inactivation of bacteria, fungi and viruses on the vast epithelial surfaces that line the respiratory, gastrointestinal and urogenital tracts. The potent antimicrobial activity of these cationic polypeptides, which is often synergistic and can be additive, provides the highly effective mechanism of action of the innate immune system. Recently, it has begun to be appreciated that the commensal microorganisms living on these mucosal surfaces stimulate epithelial cells to produce these effectors of innate immunity and thus contribute to host maintenance of defensive mucosal barriers and homeostasis. This latter point is particularly relevant to otitis media (OM) because OM is not caused by highly virulent microorganisms; OM is instead caused by a subset of the commensal bacteria that typically comprise the normal flora of the pediatric nasopharynx (NP). However, when host airway defenses are compromised, these bacteria can behave as opportunistic pathogens and gain access to the now poorly defended middle ear. It is well known that colonization of the NP is thus the very first step in the disease course of OM. Immediately preceding invasion of the middle ear, the bacterial load in the nasopharynx increases significantly. Moreover, otitis prone children are more heavily colonized by the bacterial species that are predominant in acute and chronic OM compared to their non-otitis prone counterparts, thus reinforcing the notion that maintaining a relatively low bacterial load in the NP is important to the health of the middle ear, particularly during times of viral compromise. It is highly likely that maintenance of the bacterial load in a child's NP at a noninfectious "colonizing" level is regulated on a day-to-day basis primarily by the innate immune system, including action of the APs and natural antibodies present in airway secretions, and not by effectors of acquired immunity. The relative effectiveness of the innate immune system in a given individual would then influence otitis proneness as well. Conversely, dysregulation of innate defense mechanisms (i.e. due to concurrent upper respiratory tract (URT) viral infection or genetic predisposition to an inadequate or hypo-responsive innate immune system) may allow an increase in bacterial load to a more 'infectious-prone' level. It is thereby important to define and study the innate immune system of the uppermost airway, which includes the nasopharynx, Eustachian tube and middle ear. Toward this goal, in the present application we propose to characterize and study the APs produced in the uppermost airway. Specifically, we wish to investigate their role in bacterial colonization of the nasopharynx and determine if their expression is up- or down-regulated in response to the predominant viral and bacterial pathogens of OM. Moreover, we propose to determine their relative level of expression during induction and resolution of experimental OM using well-established chinchilla models. Finally, we will deliver isolated recombinant or synthetic chinchilla APs (including the recently identified chinchilla beta defensin-1) to the uppermost airway of the chinchilla host in an attempt to inhibit the development of OM that occurs in this model.



Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R03DC005700-01  
**Program Director:** PLATT, CHRISTOPHER  
**Principal Investigator:** CAREY, JOHN P MD  
**Title:** Vestibular Recovery after Intratympanic Gentamicin  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/08/01-2005/07/31

DESCRIPTION (provided by applicant): This project will expand on the work of my NIDCD K23 Project, "Vestibular Effects of Intratympanic Gentamicin." We have found that human subjects who have intratympanic gentamicin treatment for Meniere's disease have a decline in the angular vestibulo-ocular (AVOR) reflex gain for rapid head rotations that excite the treated labyrinth. Some of these subjects have shown increased gains on the same test at later times, suggesting either recovery of peripheral vestibular function or central augmentation of gain. We have also recorded the responses of vestibular afferents in chinchillas after the identical intratympanic gentamicin treatment. This causes a profound loss of sensitivity to vestibular stimulation, but spontaneous afferent discharge is preserved. This suggests that hair cells may be only partially damaged, such that they can release neuro-transmitter to elicit the baseline activity of afferents but that their apical structures cannot transduce head acceleration. These findings correlate well with recent histologic evidence that mammalian hair cells exposed to gentamicin may shed their apical structures but maintain their basal ones (Zheng et al., 1999). The first goal of this project is to determine if the afferent processes contacting vestibular hair cells and the synaptic specializations of these hair cells are preserved after intratympanic gentamicin treatment in chinchillas using light and transmission electron microscopy. If such structural preservation is seen, it may provide a basis for the recovery of vestibular function noted in our human subjects. The second goal is to determine if central augmentation of gain occurs after intratympanic gentamicin treatment. This will be accomplished using direct galvanic stimulation of the VIIIth nerve after intratympanic gentamicin treatment. The nystagmus elicited by excitation of the nerve on the treated side should show an increased velocity compared to the control side if there is a central augmentation of gain. Understanding whether vestibular function partially recovers after intratympanic gentamicin treatment has important implications for the treatment of Meniere's disease, as preservation of function may become an important goal if vertigo remains controlled. Furthermore, the mechanisms of vestibular recovery may help us understand the potential for the inner ear to recover from a variety of injuries.

**Grant:** 1R21DC005564-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** GOLDSTEIN, RICHARD N PHD BIOLOGY NEC:BIOL  
NEC-UNSPEC  
**Title:** Role of sialylated LPS in H. influenzae otitis media  
**Institution:** BOSTON MEDICAL CENTER BOSTON, MA  
**Project Period:** 2002/04/25-2004/03/31

DESCRIPTION (provided by applicant): Nontypable Haemophilus influenzae (NTHi) is a major cause of acute otitis media (AOM) accounting for 20-30% of all episodes. There is also evidence that NTHi is the most frequent pathogen in children with recurrent episodes of AOM. The extensive heterogeneity of outer membrane proteins has been an impediment to identifying effective vaccine candidates against NTHi. In animal models, membrane protein antigens tend to induce homologous, but not heterologous protection. To date, the PI's research on H. influenzae has exclusively focused on phylogenetic characterization of species natural population structure and its use to survey degree of conservation of potential outer membrane protein targets. Because few essential virulence factors have been identified in the pathogenesis of AOM caused by NTHi, the PI carried out a preliminary pilot study to test the hypothesis that sialic acid, a terminal sugar of NTHi lipopolysaccharide, might be an essential virulence factor in AOM, analogous to the virulence role of sialic acid of LPS in gonococcal and meningococcal infections. These studies capitalized upon the availability of: (i) isogenic pairs of wild-type and LPS sialylation deficient mutants; (ii) the chinchilla animal model for experimental otitis media and (iii) the fine structural analysis of LPS, including data on organisms obtained ex-vivo from the animal model. This pilot study revealed attenuation of infection by the sialic acid deficient mutant strains compared to infection with wild-type organisms. This is consistent with prior studies showing that sialic acid deficient mutants of NTHi are relatively susceptible to complement mediated bactericidal killing by pooled human sera. The current R21 proposal aims to expand this preliminary evaluation of the role of sialic acid in the pathogenesis of AOM. It amalgamates population biology, a validated animal model of AOM, microbial genetics, and structural analysis to determine the role of sialic acid as virulence factor. These data will pave the way for further studies that could be supported by a future R01 application.

**Grant:** 1R21DC005845-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** ZENG, MINGTAO PHD  
**Title:** Genetic immunization against pneumococcal disease  
**Institution:** UNIVERSITY OF ROCHESTER ROCHESTER, NY  
**Project Period:** 2002/09/20-2005/08/31

DESCRIPTION (provided by applicant): *Streptococcus pneumoniae* is the most common bacterial cause of otitis media and acute respiratory infection and is estimated to result in over three million deaths in children every year worldwide from invasive diseases such as pneumonia, bacteremia, meningitis, and septicemia. The low efficacy of currently licensed pneumococcal polysaccharide vaccine has necessitated research into more efficient vaccines against pneumococcal disease. The long-term goal of this research is to develop a multi-component vaccine against pneumococcal disease, using genetically and antigenically conserved outer membrane proteins PspA, PsaA, and detoxified pneumolysin (PdB) from *S. pneumoniae*. Our hypothesis is that an effective pneumococcal vaccine should be composed of multiple conserved relevant antigens delivered preferably by a mucosal route in order to provide the best non-serotype-dependent protection against *S. pneumoniae* infection. Intranasal and transcutaneous immunization with replication-incompetent adenoviral vectors have proved to be efficient and simple for immunization. This non-invasive vaccine delivery will undoubtedly enhance the compliance of a vaccination program. In this project, adenovirus and plasmid expression vectors encoding PspA, PsaA and PdB will be constructed. In order to obtain an optimal vaccination protocol, immunization regimens with different combinations of adenoviral vectors through the intranasal and transcutaneous delivery modes will be compared with the intramuscular injection of plasmid expression vectors. The specific aims of this project are: Specific Aim#1: To develop a replication-incompetent adenovirus-vectored vaccine against *Streptococcus pneumoniae*. Specific Aim #2: To compare the mucosal and systemic immunity elicited by adenovirus-vectored vaccine through intranasal and transcutaneous administrations with that elicited by plasmid expression vectors through intramuscular injection.

**Grant:** 2R01DE006127-19A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** MARQUIS, ROBERT E  
**Title:** Acid/Base Physiology of Oral Streptococci  
**Institution:** UNIVERSITY OF ROCHESTER ROCHESTER, NY  
**Project Period:** 1996/08/01-2007/02/28

Dental caries continue to be a major disease in the world today with growth due to increasing world population and greater availability of disposable income in developing countries allowing for greater purchases of sweets. Even in the United States, there is a problem of some 80% of the caries in 20% of the population and the disease become lifelong now that older Americans are retaining their teeth while at the same time having reduced salivary flow become of the medicines they take. Moreover, there are indications locally that caries in children is increasing, albeit slowly. Overall, caries appears not to be under control, even in industrialized countries. The best control strategies for caries involve reducing the virulence of the pathogens. For organisms such as mutans streptococci, control can depend on inhibition of oral attachment, mainly to plaque, or reductions in acid tolerance. Since caries occurs mainly at low pH values, virulence is closely related to acid tolerance, which appears to be set mainly by F(H<sup>+</sup>)-ATPases, specifically by amounts of the enzyme per cell and pH-activity profiles of the enzymes of particular bacteria. This application is focused on acid tolerance and F-ATPases. It proposes continuation of ongoing research but also addresses new views of the multi-organism etiology of caries, of fatty- acid modulation of membrane enzymes and of substrate limitation of F- ATPase activities. The specific aims are: 1. Further studies of the F- ATPases of oral bacteria with orientation to defining the molecular bases for differences in acid tolerance among the enzymes of organisms with high and low tolerance and to evaluation of physiological controlling factors for the F-ATPase activities of the organisms, including ATP supply and associations with membrane lipids. Modulation of transmembrane transport systems by membrane fatty acids will be investigated also; 2. Studies of the acid-base physiology of acid-tolerant strains of oral streptococci selected from plaque through growth in acidified media. The work will include studies of the F-ATPase of the organisms, buffer capacities of cells to the organisms compared with mutans streptococci, responses to fluoride and other weak acids, capacities to develop acid-adaptive response, and other factors that may allow for glycolytic acid production at low pH values.

**Grant:** 2R01DE009761-11A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** TRAVIS, JAMES  
**Title:** BACTERIAL PROTEINASES IN PERIODONTAL DISEASE  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 1991/08/01-2007/06/30

DESCRIPTION (provided by applicant): A primary organism involved in the development and progression of periodontal disease is the periodontopathogen, *Porphyromonas gingivalis*. This bacterium utilizes an arsenal of virulence factors to avoid host defense, allowing for its growth and proliferation at infected sites. Among these factors are proteolytic enzymes, which appear to be involved in the deregulation of cascade pathways, the inactivation of plasma proteinase inhibitors, and the disruption of chemotactic processes, all to the benefit of the invading organism. Many of these enzymes have been isolated and characterized in this laboratory, and it seems clear that inhibitors developed against them would significantly reduce the pathological symptoms associated with periodontitis. Nevertheless, in some types of periodontal disease, especially its early phase, other bacterial pathogens are likely to be involved. For these reasons, the Specific Aims of this project are as follows: 1) to expand the model we have developed which directly points to major roles for *P. gingivalis* proteinases in the pathological hallmarks of periodontal disease (bleeding on probing, swelling, tissue resorption, bone loss), 2), to initiate experiments to determine if other bacterial pathogens which are found at the early stages of periodontal disease also utilize proteolytic enzymes for host defense evasion, and 3) to screen for and purify low and high molecular weight proteinase inhibitors which may be synthesized by other oral organisms for the possible control of *P. gingivalis* growth and proliferation. Our long-term goals are to determine whether the mechanisms used by *P. gingivalis* to avoid host defense are a general process that other pathogens have adopted and to develop inhibitors against such enzymes in order to control or eradicate bacterial infections.

**Grant:** 2R01DE011443-05A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** PASTER, BRUCE J PHD  
**Title:** Biologic Basis of Refractory Periodontal Disease  
**Institution:** FORSYTH INSTITUTE BOSTON, MA  
**Project Period:** 1997/04/01-2007/04/30

**DESCRIPTION:** The long-term goal of this research is to be able to appropriately diagnose and successfully treat all patients with periodontal disease. Currently, about 15 percent of periodontitis patients fail conventional therapy and are deemed "refractory." This application examines the hypothesis that refractory disease is distinct from non-refractory periodontal disease, and seeks means for prospectively identifying these patients. These objectives are addressed in 4 Specific Aims. Aim I will test the hypothesis that subjects with refractory disease can be distinguished from subjects with treatable periodontitis and periodontal health based upon microbial profiles. Subgingival plaque samples will be taken from 28 sites from at least 80 subjects in each of the 2 diseased groups and 40 healthy subjects. The samples will be analyzed individually for levels of approximately 120 taxa using checkerboard DNA hybridization assays. Herpes viruses will also be monitored. The SIGNIFICANCE of this Aim is that it will identify those species (both cultivable and presently uncultivable) that are useful for distinguishing between refractory and treatable periodontitis, and health. Aim 2 will test the hypothesis that subjects with refractory disease can be distinguished from those with treatable periodontitis, and periodontal health based upon host factors, such as serum antibody levels to 40 specific periodontal bacteria and cytokine production by peripheral blood monocytes following stimulation. The SIGNIFICANCE of this Aim is the identification of key host markers that differentiate between disease groups and health. Aim 3 will develop a Human Oral Microbe Microarray for the detection of 600 cultivable and uncultivable oral species found in the human oral cavity. The microbial samples from Aim 1 will be reanalyzed using the essentially complete microbial coverage provided by this microarray. This microarray will facilitate other research efforts in oral ecology, infectious diseases, and clinical studies. The microarray has obvious diagnostic value. Aim 4 will use the combined clinical, microbial and host data to differentiate refractory periodontitis from treatable periodontitis or health, and to identify refractory syndromes. Completion of this project will fill in major gaps in knowledge of the role of the total oral flora in refractory and treatable periodontitis, and of the role of cytokines, chemokines and their receptors. Eliminating refractory disease would have an enormous impact on the total cost of delivering periodontal therapy.

**Grant:** 2R01DE011831-05A2  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** HERZBERG, MARK C DDS CLINICAL  
DENT:PERIODONTIA  
**Title:** Innate Intraepithelial Defense Against Oral Infection  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 1997/01/06-2007/06/30

DESCRIPTION (provided by applicant): It is unclear if epithelial cells can defend against invading bacteria. During years 01-04, calprotectin (S100A8/S100A9; MRPB-MRP14) expression was shown to reduce invasion into epithelial cells by *L. monocytogenes*, *S. typhimurium*, and *P. gingivalis*. Resistance was associated with: i) increased  $\alpha 3$ -integrin expression; ii) altered actin organization, and iii) intracellular antimicrobial activity. Calprotectin is posited to be a multifunctional protein complex that reduces bacterial invasion by several mechanisms. Aim 1 will express defined mutants of calprotectin complex in KB cells and analyze for altered function. Site-directed mutational analysis and deletion analysis will identify structural features of calprotectin required for inhibition of invasion. Aim 2: Regulate MRP8 (S100A8) and MRP14 (S100A9) expression in KB cells using an inducible mammalian expression system. Experimental invasion will occur: 1) before cytoplasmic expression of calprotectin, 2) during half-maximal expression, and 3) when calprotectin is maximal. Calprotectin will be localized from the site of synthesis to other cell compartments over time in the presence and absence of invasion. These studies will show the staged movement of calprotectin within the cell, the relationship to the invasion of different species, and whether bacteria actually invade before inhibition by calprotectin. Aim 3: Target calprotectin to selected subcellular locations to enhance or reduce its anti-invasion action. Calprotectin will be over-expressed in selected subcellular and extracellular compartments and under-expressed in the cytoplasm. Cells over-expressing membrane and secreted calprotectin (under-expressed in the cytoplasm) will be compared to normal calprotectin-expressing cells for anti-invasion and anti-microbial activity. If cytoplasmic interactions were crucial, membrane or secreted calprotectin would be ineffective in inhibiting invasion. AIM 4: Compare human gingival epithelial cells and KB cells for the effect of calprotectin on bacterial invasion. Human gingival epithelial cells will be incubated with PMA to up-regulate calprotectin, or retinoic acid, DMSO or vitamin D3 to down-regulate expression. After dose-response optimization, and comparison to KB cells to identify non-specific changes in invasion phenotype, calprotectin will be localized. Aim 4 will bridge molecular mechanisms of innate resistance to bacterial invasion in stable transfectants to normal epithelial cells in vitro. These Aims will show the multifunctional potential for calprotectin to make mucosal epithelial cells resistant to bacterial invasion in vitro, suggesting new mechanisms of innate immunity in vivo and functional targets for new anti-bacterial therapies.



**Grant:** 2R01DE012236-07  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** BURNE, ROBERT A  
**Title:** GENE REGULATION AND PHYSIOLOGY OF STREPTOCOCCUS MUTANS  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 1997/04/01-2007/04/30

DESCRIPTION (provided by applicant): Dental caries remains among the most prevalent infectious diseases worldwide. Over 84 percent of U.S. children, 96 percent of U.S. adults, and 99.5 percent of Americans 65 years of age and older have experienced tooth decay. To conceptualize and develop novel anticaries strategies that can be effectively distributed to the population, a molecular dissection of the genetics, physiology and biochemistry of cariogenic microorganisms is needed. Our work during the previous funding period has led to the discovery of novel and unique mechanisms for the control of the degradation of dental plaque polysaccharides and revealed important connections between the amount of carbohydrates available to cells and the capacity of these cells to express essential virulence determinants, including the capacity to produce exo-polysaccharides and to tolerate acidic conditions. Further, our work has led to the discovery that global regulators of carbohydrate metabolism play essential roles in regulation of virulence and expression of acid tolerance. The major goals of this application are to continue with our fundamental studies on gene regulation and physiology of *S. mutans*, with a particular focus on the control of virulence gene expression by carbohydrate availability, pH and the global control protein CcpA. To accomplish these goals, we have established the following four Specific Aims. 1. A detailed analysis of a transcriptional activator required for fructanase (*fruA*) expression and analysis of the molecular basis for PTS-mediated control of expression of fructan degradation. 2. Identification of the protein(s) that interacts with the *fruA* catabolite response elements. 3. Molecular dissection of the basis for the control of exo-polysaccharide production by carbohydrate availability and CcpA. 4. Physiologic and genetic analysis of the linkage between limitation for carbohydrate, the CcpA regulon and control of virulence gene expression. These studies combine sophisticated molecular genetic tools with powerful physiologic techniques to dissect the pathways used by *S. mutans* to alter its pathogenic potential in response to carbohydrate availability. Understanding these pathway will allow for the design of technologies that subvert the capacity of *S. mutans* to become a numerically significant constituent of a cariogenic microflora.

**Grant:** 2R01DE012505-05  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** LAMONT, RICHARD J PHD VET  
MEDICINE:MICROBIOLOGY  
**Title:** Molecular Aspects of Oral Plaque Formation  
**Institution:** UNIVERSITY OF FLORIDA SEATTLE, WA  
**Project Period:** 1998/09/01-2007/08/31

DESCRIPTION (provided by applicant): Dental plaque develops as a complex biofilm on the tooth surface and is a direct precursor of periodontal diseases, one of the most common bacterial infections in developed countries. The general principles that cause the transformation of a commensal plaque biofilm into a potentially pathogenic entity include binding to the tooth surface by early colonizers such as oral streptococci, which then provide an attachment substrate for the subsequent colonization and biofilm formation by periodontal pathogens such as *Porphyromonas gingivalis*. Co-adhesion between *S. gordonii* and *P. gingivalis* is thus considered to be an important factor that facilitates the persistence of *P. gingivalis* in the oral cavity. Investigations in our laboratory have revealed that this adherence interaction is multimodal, involving several interacting adhesin and receptor molecules. Adhesins identified to date include the *P. gingivalis* major and minor fimbriae, the latter recognizing the SspB protein on the *S. gordonii* cell surface. Binding of SspB is dependent upon a structural binding domain conferred by amino acid residues asparagine at position 1182, and valine at position 1185. This minor fimbriae-SspB interaction provides the impetus for the formation of a *P. gingivalis* biofilm. The objectives of this application are: to define the structural requirements of the *S. gordonii* SspB receptor; to define the functionality of the *P. gingivalis* minor fimbrial adhesin; to identify the *S. gordonii* cognate receptor(s) for the *P. gingivalis* major fimbrial protein; and to determine differential expression of *P. gingivalis* genes induced by adhesin-receptor interactions. These Aims will thus provide a more detailed molecular picture of the components responsible for *P. gingivalis*-*S. gordonii* co-adhesion and their mechanisms of action. Furthermore, we will begin to appreciate the pathways and circuitry by which co-adhesion leads to biofilm development. The information provided by these studies will enhance our understanding of how pathogenic and commensal plaque bacteria interact on a cellular and molecular level during the process of colonization and biofilm development. Such insights will provide a knowledge base that will facilitate the development of novel methods to control periodontal disease based on inhibition of colonization mechanisms or interference with the regulatory mechanisms that control the expression of adhesins or the accumulation of biofilms.

**Grant:** 1R01DE013664-01A2  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** FLETCHER, HANSEL M PHD IMMUNOLOGY AND MICROBIOLOGY  
**Title:** Studies on virulence regulation in Porphyromonas  
**Institution:** LOMA LINDA UNIVERSITY LOMA LINDA, CA  
**Project Period:** 2002/04/01-2006/02/28

*Porphyromonas gingivalis*, a black-pigmented, gram-negative anaerobe, is widely implicated as an important etiological agent of periodontal disease. This bacterium expresses several potential virulence factors (e.g., capsule, LPS, fimbriae, membrane vesicles, and hydrolytic enzymes) that may contribute to its pathogenicity. Another virulence factor, the *recA* gene, confers resistance to the oxidative stress environment of the inflammatory periodontal pocket. The *recA* gene product is a key protein in DNA repair that protects *P. gingivalis* from DNA damage induced by bactericidal reactive oxygen derivatives generated in the periodontal pocket by neutrophils and transient air exposure. Our laboratory has identified two genes, *vimA* and *bcp*, that may be part of the *recA* transcription unit and may also function in virulence. Further, the *vimA*-mediated virulence modulation in *P. gingivalis*, may represent a novel posttranscriptional regulation of virulence factors in this organism. Because the BCP homologue may have peroxidase function, and gingipains are involved in heme accumulation which can inactivate H<sub>2</sub>O<sub>2</sub>, it might be considered an important strategy for the organism to coordinate its oxidative stress and proteolytic activities. This importance is further supported by observation that the *recA* locus promoter is active during infection of the murine host. Moreover, the promoter activity is affected by temperature, iron and calcium which are factors known to coordinately regulate the expression of other bacterial virulence genes. Our observations, taken together, may suggest an important role for the complex *recA* locus in the survival and virulence of *P. gingivalis*. It is our hypothesis that the *bcp-recA-vimA* transcriptional unit is important for virulence and protection against oxidative stress. Our overall objective is to elucidate the molecular mechanism(s) for the *vimA*-mediated virulence regulation and examine the relative importance of the *bcp-recA-vimA* operon in oxidative stress resistance in *P. gingivalis*. Specific aims for the proposed research are: 1) To characterize the *bcp-recA-vimA* transcriptional unit in *P. gingivalis* W83. This will include: a) mapping the transcription initiation site; b) verifying the promoter sequence upstream of the primary start site; c) evaluating the effect of the *bcp* gene on the function on the *recA* and *vimA* genes; 2) To examine the functional significance of the *vimA* mutation on protease activation in *P. gingivalis* W83; and 3) To evaluate the importance of the *bcp-recA-vimA* transcriptional unit in oxidative stress protection.

**Grant:** 1R01DE013937-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** CAUFIELD, PAGE W DDS  
**Title:** Microbial Diversity and Genetic Characterization  
**Institution:** NEW YORK UNIVERSITY NEW YORK, NY  
**Project Period:** 2002/06/15-2005/05/31

The proposed studies are anticipated to yield important new information as to the composition of the bacterial biota that comprises the caries-associated plaque biofilm. The long-term benefit of such information should lead researchers to devising both diagnostic and preventative strategies for dental caries based on addressing its etiological agents. This proposal will focus on children with a severe forms of dental caries called Early Childhood Caries (ECC). Using a powerful technique of gradient electrophoresis will be used to separate 16S rDNA markers from an array of bacteria in plaque biofilms. These gels should show differences between the microfloras of ECC and caries-free children. This profiling, in turn, will allow us to identify or approximate those bacteria, some likely to be uncultivable, associated with caries. Another hypothesis to be tested is whether strains of mutans streptococci, or the entire caries-biofilm differ in their ability to cause disease. Subtraction DNA hybridization will be used to discover unique genetic loci present in mutans streptococci or dental plaques of caries-prone children. Further development of subtraction DNA hybridization will lead to our overall objective, i.e., to characterize from whole plaque a constellation of genetic loci within the caries-active biofilm, irrespective of the limitation of first cultivating specific bacteria. This will set the groundwork for subsequent studies in which a set of DNA probes can be compiled and tested, which will be useful for predicting whether a particular child is at risk for caries. Knowing the function of these genetic loci and the bacterial host from which they arise will give important information as to the causation of caries. Moreover, having genetic markers for disease may eliminate the costly and imprecise practice of cultivating bacteria from dental plaque. The research proposed will likely impact on these more serious forms of caries, leading to its eventual prevention.

**Grant:** 1R01DE013957-01A2  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** PROGULSKE-FOX, ANN  
**Title:** Technology for Analysis of Porphyromonas gingivalis  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 2002/06/01-2007/04/30

DESCRIPTION (provided by applicant): Porphyromonas gingivalis is considered an important etiologic agent of adult periodontal diseases and is also implicated in some systemic diseases such as cardiovascular disease and low birth weight. The goal of this application is to apply a fundamentally new approach to identify P. gingivalis genes that are expressed during in vivo but not in vitro growth. Such genes are likely to be important to the ability of P. gingivalis to cause disease. The approach, called In Vivo Induced Antigen Technology (IVIAT), is superior to other related technologies in a number of ways, but most importantly because it does not rely on animal models to mimic the growth of the pathogen in humans. IVIAT uses antibodies present in pooled sera from infected patients as probes to identify the pathogen genes of interest. This will be accomplished in three Specific Aims. In the first Specific Aim, pooled sera from periodontitis patients will be exhaustively adsorbed with in vitro grown whole P. gingivalis cells and cell extracts. The resulting serum will be used to probe a genomic expression library of P. gingivalis in Escherichia coli using colony-blotting methods. In Specific Aim 2, the cloned DNA inserts in reactive clones will be sequenced and analyzed to determine the cloned open reading frames (ORFs) likely to be responsible for expression of the in vivo induced (IVI) antigens. The pertinent ORFs will be subcloned into an appropriate expression vector and at least 1 mg of the expressed protein will be purified to homogeneity. In Specific Aim 3, a direct proof that P. gingivalis produces IVI antigens during an actual infectious process will be performed by probing P. gingivalis cells recovered from plaque samples of infected patients with labeled antibodies specifically directed against the IVI antigens. The results of these studies are expected to improve understanding of the pathogenic mechanisms employed by P. gingivalis by identifying virulence-associated genes that would not be found by conventional methods. These genes and their products are excellent candidates for therapeutic and diagnostic targets and for vaccine design.

**Grant:** 1R01DE014191-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** SHENKER, BRUCE J PHD  
MICROBIOLOGY:IMMUNOLOGY  
GY  
**Title:** Immunosuppressive Proteins Produced by Oral Pathogens  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 2002/05/01-2007/02/28

DESCRIPTION (provided by applicant): Over the past several years, significant progress has been made in understanding of the etiology and pathogenesis of periodontal diseases. Nevertheless, the nature and contribution of the immune system to these disorders remain unclear. The basic hypothesis is that the immune system plays a primary role to minimize and/or prevent infection. Furthermore, the application posits that immunoregulatory abnormalities contribute to the pathogenesis of and susceptibility to periodontal disease. In this regard, the prior investigations have demonstrated that *Fusobacterium nucleatum* and *Treponema denticola* produce immunosuppressive proteins (ISPs). The fundamental hypothesis of the proposed studies is that periodontal pathogens produce ISPs that mediate local and/or systemic immunosuppression, thereby enhancing their own virulence and/or that of other opportunistic microorganisms. The plan is to focus this investigation on the *F. nucleatum* (Fip) and *T. denticola* (Sip) ISP which has been shown to induce human lymphocytes to arrest in the mid G1 phase of the cell cycle. Moreover, the preliminary studies determined each ISP is composed of two subunits. The objectives of this application are to define the events responsible for ISP-induced G1 arrest and to determine the relationship between structure and function of the ISP subunits. The study is composed of four Specific Aims: 1) To determine the molecular mechanism(s) responsible for *F. nucleatum* (Fip) and *T. denticola* (Sip) ISP-induced G1 arrest in human lymphocytes; 2) To determine if G1 arrest is irreversible resulting in activation of the G1 checkpoint and the apoptotic cascade; 3) To determine if Fip exists and functions as a heterodimer and examine the individual role of Fip A and Fip B in the induction of G1 arrest; and 4) To determine if the two peptides that comprise the ISP of *T. denticola* (Sip) are encoded by separate genes and, if so, to determine the functional role of each peptide.

**Grant:** 1R01DE014198-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** REYNOLDS, ERIC C PHD  
**Title:** Development of a P.gingivalis vaccine  
**Institution:** UNIVERSITY OF MELBOURNE MELBOURNE,  
**Project Period:** 2002/07/01-2007/04/30

DESCRIPTION (provided by applicant): Periodontal diseases are bacterial-associated inflammatory diseases of the supporting tissues of the teeth and the more aggressive forms, periodontitis, are characterized by the destruction of the tooth supporting structures. Current treatments for the periodontal diseases are nonspecific and are based on the continued removal of subgingival plaque by mechanical means, often involving surgical procedures. This ongoing therapy is costly and has a variable prognosis due to patient non-compliance. Recent evidence has demonstrated that the bacterium *Porphyromonas gingivalis* is a major etiologic agent of some forms of these diseases. The broad objective of this application is to determine the suitability of the major outer membrane proteins of *P. gingivalis*, that the earlier studies by identified candidates for the development of a defined recombinant or synthetic vaccine. In the long-term, this may lead to the development of effective, specific and novel prophylactic and therapeutic strategies for *P. gingivalis*-associated periodontitis. Over thirty outer membrane proteins of *P. gingivalis* have been developed and six of these will make excellent candidates for vaccine development. Specifically, a preparation will be used to prepare these selected *P. gingivalis* outer membrane proteins and protein fragments using a bacterial expression system and determine the immune response to these purified recombinant proteins in mice. A further Aim is the preparation of synthetic peptide multivalent constructs based on immunogenic sequences of the outer membrane proteins. The recombinant proteins, protein fragments and synthetic peptide multivalent constructs will be tested as defined vaccines against *P. gingivalis* in murine models of disease.

**Grant:** 1R01DE014360-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** SOUKOS, NIKOLAOS S  
**Title:** Photosensitization of Oral Bacteria  
**Institution:** FORSYTH INSTITUTE BOSTON, MA  
**Project Period:** 2002/09/15-2005/08/31

DESCRIPTION (provided by applicant): The overall goal of this research is to develop a clinically useful way to enhance the penetration and effectiveness of photosensitizers (PS) in human Dental plaque for prevention and treatment of periodontitis. The hypotheses to be tested are: (a) Laser-generated photomechanical waves (PW) can induce in vitro enhanced permeabilization of oral bacterial biofilms of different density to positively charged PS, such as a polycationic conjugate between poly-L-lysine (p1) and the PS chlorin e6 (ce6) and the cationic methylene blue (MB), an approach which can significantly increase uptake of ce6 and MB by bacteria, and (b) Photodestruction of these bacterial biofilms in vitro may be possible after their sensitization with the pl-ce6 conjugate and MB followed by exposure to PW and red light. The interaction of the PS with biofilms of periodontopathogens will be studied using two in vitro models: a) monospecific biofilms growing attached to natural enamel surfaces, and b) multispecific biofilms growing on natural enamel surfaces in a chemostat system. The uptake of PS will be quantitated by measuring fluorescence and their local interaction with bacteria and the biofilm matrix will be evaluated by confocal scanning laser microscopy. Laser-induced PW will be applied in the presence of the PS to disorganize the biofilm structure and increase their penetration. Other disorganizing agents of the bacterial biofilm will be used for comparisons. In all cases, the penetration of the PS will be evaluated by confocal scanning laser microscopy. In the same biofilm models the photodynamic effects of the PS will be established and PW-enhanced photosensitization of periodontopathogens by the PS will be evaluated.



**Grant:** 1R01DE014368-01A1

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

**Principal Investigator:** SOCRANSKY, SIGMUND S

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UNSPEC

**Title:** Intra-Oral Biofilm Formation

**Institution:** FORSYTH INSTITUTE

BOSTON, MA

**Project Period:** 2002/09/15-2005/08/31

DESCRIPTION (provided by applicant): The major problems that affect the oral cavity are caused by organisms that live in biofilms colonizing the hard and soft tissues. While much has been learned about mature Dental plaque, little is known about the sequence of changes in microbial species that occurs during supra or subgingival plaque development. Even less is known about the soft tissue microbiota and the interplay between soft tissue microbiota and hard tissue microbiota during biofilm development. Two important bulk fluids, saliva and GCF, bathe the biofilm surfaces and play an undetermined role in biofilm development. Thus, the objectives of this application are to define the changes in microbial species that occur during early biofilm re-development and to evaluate factors that might influence this development including diminished salivary flow, lack of natural dentition and changes in gingival inflammatory status. SA1 will compare the changes in microbial composition of developing biofilms on hard and soft tissues in 60 periodontally healthy, 60 periodontitis, and 60 denture-wearing, edentulous subjects from initial Dental cleaning to 7 days. Supra and subgingival plaque samples will be taken separately from the mesial aspect of each tooth at baseline and supragingival samples from dentures. Saliva and samples from eight oral soft tissue surfaces will also be collected. Samples will be evaluated individually for their content of 40 bacterial species using checkerboard DNA-DNA hybridization. After initial monitoring, all dentate subjects in each group will receive a Dental cleaning while dentures will be cleaned and disinfected. Thirty randomly selected subjects in each group will receive full mouth "soft tissue cleaning and disinfection. Subjects will refrain from oral hygiene for 7 days during which time Supra and subgingival plaque samples will be taken from randomly selected quadrants at 1, 2, 4, and 7 days. Denture plaque samples will be taken at the same points as saliva and soft tissue samples. SA2 will compare the microbial composition of supra and subgingival plaque, oral soft tissues, and saliva in 60 Sj\_gren's subjects and 60 matched controls. Full-mouth caries and periodontal status will be assessed and samples of supra and subgingival plaque will be taken from the mesial aspect of each tooth at baseline. In addition, eight soft tissue samples and a saliva sample will be taken. All samples will be evaluated microbiologically as described above. All subjects will receive full mouth SAP and will be re-assessed microbiologically at 28 days. The proposed study should indicate the nature of the microbial changes that occur during biofilm development on hard and soft tissues and the influence that saliva, GCF and the natural dentition have on the developing biofilms.

Includes Research Project Grants (RPGs)

Excludes Clinical Trials

**Grant:** 1R01DE014713-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** FIGURSKI, DAVID H PHD  
**Title:** Tight Adherence Genes of a Periodontal Pathogen  
**Institution:** COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY  
**Project Period:** 2002/09/01-2007/07/31

DESCRIPTION (provided by applicant): The Gram-negative bacterium, *Actinobacillus actinomycetemcomitans*, is believed to be the etiologic agent for localized juvenile periodontitis (LJP), a particularly destructive disease in adolescents. The incidence of LJP varies among demographic groups and disproportionately burdens minorities and the poor. *A. actinomycetemcomitans* has been associated with a variety of other infections, notably brain abscesses, and it is a member of the clinically important HACEK group of bacteria implicated in infective endocarditis. A striking characteristic of fresh clinical isolates of *A. actinomycetemcomitans* is their ability to form extremely tenacious biofilms, a property thought to be critical for colonization of teeth and other surfaces. Molecular genetic studies in this laboratory have revealed that the genome of *A. actinomycetemcomitans* maintains a cluster of *tad* genes required for tight adherence to surfaces. The studies indicate that the *tad* genes are part of a locus of 14 genes encoding a novel secretion system for the assembly and release of long, bundled FliP fibrils and that the fibrils are required for tight nonspecific adherence to surfaces and bacterial autoaggregation. Remarkably similar *tad*-like loci were subsequently found in the genome sequences of a wide variety of Gram-negative and Gram-positive Bacteria, including many significant pathogens, and in Archaea. Given the clear requirement of the *tad* gene cluster for adherence of *A. actinomycetemcomitans*, the *tad* loci in other organisms are likely to be important for microbial colonization in a variety of environmental niches. Proposed here are molecular and genetic studies of the *tad* locus of *A. actinomycetemcomitans*. The objectives are the following: 1) to understand the mechanisms of expression and regulation of the genes of the *tad* locus; and 2) to determine the locations, interactions, and molecular functions of the gene products in secretion and fibril assembly. These studies are expected to lead to a basic understanding of a novel secretion system of bacteria and its role in the biogenesis of fibrils required for tight adherence and colonization by *A. actinomycetemcomitans*. Since of the widespread nature of the *tad* loci, these studies should also lead to new insights into colonization by other bacterial pathogens and may serve to identify new targets for development of antibiotics.

**Grant:** 1R01DE014819-01  
**Program Director:** NOKTA, MOSTAFA A  
**Principal Investigator:** XIE, HUA MS  
**Title:** Inhibition of HIV Infection by Oral Bacterial Component  
**Institution:** MEHARRY MEDICAL COLLEGE NASHVILLE, TN  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): HIV-1 infection is associated with the development of acquired immunodeficiency syndrome (AIDS), a devastating disease affecting over 40 million people worldwide. Oral transmission of HIV appears to be a rare event, compared to a much higher risk of vaginal and rectal transmission. In search for the explanations for this phenomenon, intensive studies have focused on identification of oral components that are responsible for inhibition of HIV oral transmission. In our ongoing studies, we have demonstrated that *Porphyromonas gingivalis*, a periodontopathogen detected in most disease sites of periodontitis patients, possesses the ability to inhibit HIV induced membrane fusion. Our long-range goal is to fully understand the involvement of oral bacteria in the oral transmission of HIV and to provide the basis for development of effective anti-HIV therapies. The hypothesis for this proposal is that *P. gingivalis* is a potent oral inhibitor of HIV transmission, and that the primary mechanism of the inhibition is that a *P. gingivalis* component specifically interferes with one of the key steps in the early stage of HIV infection. To test the hypothesis, we will start with identification and purification of the inhibitory molecule. The inhibitory molecule will be characterized in the term of functional and genetic structures. We will also attempt to elucidate the molecular target of the *P. gingivalis* inhibitory component. Experiments are designed to examine every possible event where the bacterium steps in and leads to an aborted viral entry. Special effort will also be made to evaluate the clinical potential of the inhibitory molecule by determining its anti-viral spectra. Finally, studies will be initiated to investigate the effect of co-infection of HIV and *P. gingivalis* on the pathogenicities of the organisms. The successful completion of the proposal will be a significant advance in the understanding of the role of *P. gingivalis* in HIV oral transmission. Furthermore, the study of *P. gingivalis* inhibitory protein may lead to a discovery of an additional target to complement the current anti-HIV targets which are either reverse transcriptase or protease.

**Grant:** 1R01DE014897-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** DIAMOND, GILL PHD ORAL MICROBIOLOG  
**Title:** Bacteria-host cell interactions in periodontal disease  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2002/06/15-2006/03/31

Localized juvenile periodontitis (LJP) is a periodontal disease which primarily affects peripubertal children of African descent. Over 95 percent of the LJP patient population is colonized with the facultative Gram negative rod *Actinobacillus actinomycetemcomitans* (Aa). In LJP, virulent strains of Aa invade the epithelial cells, and colonize the oral mucosa, and induce a hyperinflammatory response. A bacterial virulence factor which encodes a secreted leukotoxin inhibits bactericidal activity by the PMNs, allowing colonization. For the initial colonization and subsequent invasion, however, the bacteria must first evade the initial line of host defense in the oral cavity. This primary part of oral innate immunity is based in the response of the epithelial cells by the production of antimicrobial agents and inflammatory mediators. Little is known about how the periodontal epithelium responds to the presence of bacteria in general, and Aa in particular. Furthermore, while LJP is a disease with a large genetic component which only manifests with localized periodontal infections, the deficiencies in the host defense capabilities of this epithelium have not been examined. Thus, a more complete analysis of the innate immune response of the gingival epithelium to Aa will allow for a better understanding of the etiology of this disease. The long-range goal of our research is to better understand the dynamic host defense systems in the mucosal epithelium. The objective of these studies is to determine how a pathogenic bacterium evades the innate immune response in individuals which are predisposed to this infection. Our central hypothesis is that the gingival epithelium provides an active host defense tissue. A combination of deficiencies in this host defense and bacterial virulence factors can lead to severe infection. By characterizing the response of the epithelial cells to the pathogen, and identifying differences in cells from diseased versus normal individuals, we will be able to better address the issues of early detection and treatment. This would include strategies to modulate the endogenous antimicrobial peptide expression to prevent serious bacterial infections. To achieve this we propose to: 1. Characterize the specific pattern recognition receptors to Aa in the gingival epithelium. 2. Define the host defense gene expression of the cultured gingival epithelium in response to Aa. 3. Determine innate immune gene expression in the oral epithelium from healthy and LJP patients. For this study we will focus on the role of pattern recognition receptors such as CD14 and Toll- like receptors, antimicrobial peptide and production of proinflammatory cytokines. Our approach, which includes studying the interactions of the epithelium with live bacteria and the use of microarrays, will provide a detailed picture of the host defense capabilities of the oral cavity. Characterization of variability in the expression of specific natural antibiotics such as beta-defensins or stimulants of neutrophil antibacterial function such as chemotactic and proinflammatory cytokines, which may prevent colonization of periodontal tissues by this persistent bacterium, may allow for early identification of the susceptible individuals or provide an alternative treatment approach for this patient category. The result from this study will allow us to develop novel strategies to prevent attachment and colonization of disease-causing bacteria.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R03DE014443-01A1  
**Program Director:** NOKTA, MOSTAFA A  
**Principal Investigator:** ERDMAN, SCOTT E PHD  
**Title:** Functions, Traffic and Targeting of Fungal Adhesins  
**Institution:** SYRACUSE UNIVERSITY SYRACUSE, NY  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): The fungal cell wall plays a critical role in protecting cells from osmotic stress and, in conjunction with the actin cytoskeleton, in regulating cell shape as a function of cell growth. In many fungal species growth is polarized during the formation of buds, mating projections and hyphae. Components found in the cell wall also regulate cell-cell adhesion reactions such as flocculation during vegetative growth, sexual agglutination and pathogen-host cell adhesion. These processes are crucial to the life cycles of many fungi pathogenic to humans including *Candida* species, *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Histoplasma capsulatum*. While many key adhesion molecules responsible for cell-cell adhesion have been identified, little is presently known concerning the factors that regulate the localization, trafficking and activity within the cell wall of these glycoproteins which play key roles in pathogenesis. This proposal aims to further elucidate the structure and function of conserved WCPL and CX4C domains found in a super family of cell wall Mann proteins that are present in both *S. cerevisiae* and *C. albicans* and involved in their differentiation. The genetically tractable system of cell adhesion during mating in *S. cerevisiae* will be used to study the domain organization, cell wall localization and mechanisms regulating activity of a model cell wall protein, Agalp, which is a member of the super family. These studies will use biochemical and genetic methods to investigate the possibility that the WCPUCX4C domains mediate post-secretory traffic of the protein within or at the surface of the fungal cell wall. A novel approach designed to develop peptide reagents with the potential to act as specific inhibitors of the fungal adhesins Aga2p and Hwpl p and/or as structural platforms for antifungal drug development and delivery will be explored. We expect these studies to add to our understanding of fungal cell wall Mann protein modification, localization and function; such information is likely to be useful to the future design and targeting of anti-fungal agents.

**Grant:** 1R03DE014452-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** ROGERS, JEFFREY D DDS  
**Title:** Antibiotic Resistance in the Oral Streptococci  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 2002/01/01-2003/12/31

**DESCRIPTION:** (provided by applicant) The continuing increase in bacterial resistance to antibiotics is a significant worldwide problem. Although most research has focused on patterns of resistance in pathogenic organisms, the importance of commensal bacteria as reservoirs of antimicrobial resistance determinants is now beginning to be recognized. We seek a better understanding of antibiotic resistance genes, particularly those conferring beta-lactam resistance, in the oral streptococci. The discovery of altered penicillin-binding proteins (PBPs) as the basis of beta-lactam resistance in *S. pneumoniae* quickly led to the hypothesis that these *pbp* genes evolved by recombination with homologs from other streptococcal species, in particular the oral streptococci. This gave rise to mosaic sequences containing sequence blocks highly divergent from those of sensitive strains that encoded novel PBPs with decreased affinity for beta-lactam compounds. The contribution of such mosaic genes to the emergence of penicillin resistance is widely accepted. However, both the source and mechanism of dissemination of sequences giving rise to mosaic genes is not fully understood. We hypothesize that the genesis of mosaic low-affinity PBPs occurs in the oral streptococci through the accumulation and exchange of point mutations in *pbp* genes, and that the oral streptococci are a reservoir of antibiotic resistance genes that are exchanged between both commensal and pathogenic microorganisms. We will use the paradigm established for the PBPs of *S. pneumoniae* as a model for molecular studies of the *pbp2x* and *pbp2b* genes of the oral streptococci. Our analysis of *pbp* genes will be carried out in oral streptococcal isolates representing the species *salivarius*. This information will be extended to mixed growth studies that will document both the genesis and direct exchange of resistance genes between the oral streptococci, including *S. pneumoniae*, thus linking genetic exchange, the genesis of antibiotic resistance genes, and the role of the oral streptococci as a reservoir of antibiotic resistance determinants.

**Grant:** 1R03DE014459-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** CHAMPAGNE, CATHERINE M PHD  
**Title:** Atherogenicity of Pathogens in Murine Model  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NC  
HILL  
**Project Period:** 2002/08/01-2004/07/31

DESCRIPTION (provided by applicant): Human epidemiological studies have shown an association between periodontal and vascular diseases. In parallel, the inflammatory -and potentially infectious- nature of atherosclerosis is gaining recognition, suggesting a potential link between infectious diseases, such as periodontitis, and atherosclerosis. However, the effect of periodontal pathogens in atherosclerosis has not been clearly established. The goal of this proposal is to investigate the contribution of oral pathogens to the development of atherosclerotic lesions in a murine model. The apolipoprotein E knockout mouse (ApoE) prone to atherosclerosis will be infected with one recognized human periodontal pathogen in an established model of chronic and localized infection (subcutaneous chamber). Experimental conditions (including power calculations) have been optimized in preliminary studies using this model of infection-enhanced atherosclerosis. Aim 1 will focus on answering the question "could periodontal pathogens contribute to the development of atherosclerotic lesion?" Localized infection will be established by intra-chamber injection of live bacterial cultures of *Porphyromonas gingivalis* in pre-immunized ApoE mice. Controls will receive medium alone. Two strains of *P. gingivalis* with varying virulence properties will be tested for their ability to induce an increase in the aorta atheroma lesion size (evaluated at sacrifice by histomorphometry). In parallel, the inflammatory response will be monitored by measuring levels of serum inflammatory cytokines (IL-6 and IL-1 by ELISA) and acute phase proteins (Serum Amyloid A by ELISA). Interaction analyses will be performed to test our hypothesis that infection with *P. gingivalis* contributes to the development of atherosclerotic lesion by triggering an inflammatory and acute phase response. Aim 2 will address the question "what specific virulence trait(s) expressed by *P. gingivalis* contribute to the development of atherosclerotic lesion?" Mutants of *P. gingivalis* for specific virulence factors (Pep0 and FimA,) will be prepared in the most atherogenic strain identified in aim 1 in laboratories. These mutants will be tested in our murine model in regard to atheroma lesion size. In addition, presence of *P. gingivalis* DNA will be investigated by PCR in descending aorta and liver samples, and serum anti-*P. gingivalis* antibody levels will be measured by ELISA, to test our hypothesis that specific virulence traits confer to *P. gingivalis* the ability to evade the host antibody defense system, and reside in organs such as the aorta and the liver. Results from these studies will help in the identification of new targets for atherosclerosis and vascular disease therapies.



**Grant:** 1R03DE014466-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** HAASE, ELAINE M BS  
**Title:** Studies of the Flp operon in *A. actinomycetemcomitans*  
**Institution:** STATE UNIVERSITY OF NEW YORK AT AMHERST, NY  
BUFFALO  
**Project Period:** 2002/01/01-2003/12/31

**DESCRIPTION:** (provided by applicant) *Actinobacillus actinomycetemcomitans* (Aa) is a gram-negative bacterium whose primary habitat in humans is the subgingival sulcus. The association between Aa and aggressive periodontitis in adolescents (e.g. localized juvenile periodontitis, LJP) provides the most compelling evidence for bacterial specificity in periodontitis. Fresh isolates with a "rough," adherent colony phenotype spontaneously and irreversibly switch to a non-adherent, smooth colony phenotype when grown in broth. Recent preliminary studies in rats found only the rough phenotype cells capable of colonizing the oral cavity. The rough phenotype is primarily associated with numerous bundle-forming fimbriae on the cell surface. These fimbriae are encoded by a fimbrial operon (the flp operon) that has recently been identified in Aa. Transposon analysis has demonstrated that this operon is important in fimbrial expression. It likely influences phase variation, biofilm formation, and possibly leukotoxin association with the bacterial cell. Although the Flp fimbriin subunit encoded by the first gene of this operon shares homology with type V-like fimbriin, it is unique in several respects, especially its small size (6.5 kb). The focus of our study on regulation will be the 5' end of the operon, the flp to tadA sequence, based on previous complementation studies in transposon mutants. One of the SPECIFIC AIMS of this proposal is 1.) to determine the molecular basis of fimbrial phase variation through the study of transcription, translation, and cell localization of Flp fimbriin subunit. By comparing the DNA sequence, transcription, and cell localization of the flp subunit in rough and spontaneous isogenic smooth variants, we will identify at what molecular level fimbrial expression is interrupted in the smooth variant, and identify potential cis regulatory sequences. Our preliminary transcriptional analysis of these genes suggests there may be more than one polycistronic message and more than one promoter within the operon. Our goal is 2.) to perform functional analysis of potential promoter regions and to determine the transcriptional organization of the flp operon through the study of selected deletion mutants. The experiments proposed in this application, when successfully completed, will provide the essential preliminary results for subsequent experiments to define the basis of fimbrial regulation in Aa. The information gained by these studies will ultimately aid in the development of novel strategies for preventing colonization of Aa and subsequent periodontal disease.

**Grant:** 1R03DE014807-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** HAHN, CHIN-LO PHD  
**Title:** Oral Streptococci, Cytokines, PAF-r in EC Coagulation  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 2002/09/01-2004/08/31

DESCRIPTION (provided by applicant): Our long-term objectives are to understand the pathophysiology of streptococcal endocarditis which causes a significant health problem especially in immune compromised patients. Its diagnosis is often not made until late in disease process and the complication is serious and the cost for treatment is expensive. The traditional view of pathogenesis suggests that injury to endothelium predispose to non-bacterial thrombotic vegetation and infective endocarditis from a bacteremia episode. However, this model does not explain the occurrence of infective endocarditis in individuals without known valvular defects. Clinically, septic shock syndrome is related to excessive cytokine induction by endotoxin which causes intravascular coagulation and multiple organ failure. Our preliminary results have shown extraordinary induction of IFN-gamma and TNF-alpha by several oral streptococcal isolates implicated in endocarditis. In addition, we observed oral streptococci invaded human umbilical vein endothelium via PAF-receptors which could be indirectly upregulated by inflammatory cytokines. These observations prompt us to hypothesize that excessive proinflammatory cytokines induced by oral streptococci might be a key host factor in bacterial invasion of endothelial cells (EC) in the heart valves. We further hypothesize that streptococcal invasion to endothelial cells via PAF-receptors causes procoagulant change in EC. Due to the limited scope of this application, we propose to study three representative oral streptococci frequently recovered from endocarditis patients - *S. mutans*, *S. oralis* and *S. sanguis*. We shall determine the streptococci-induced host cytokine effects in promoting coagulation of EC by measuring tissue factor activities. The major cytokine in tissue factor induction will be identified and the synergy among proinflammatory cytokines (IL-1, TNF-alpha and IFN-gamma) and synergy between cytokines and bacterial invasion in promoting coagulation will be examined. We will also investigate the PAF-receptor dependent adherence/invasion mechanism and its relation to coagulation. The results of this study would help to clarify or identify the role of host cytokine induced by oral streptococci in pathogenesis of streptococcal endocarditis. We also hope to provide an insight regarding streptococcal invasion into EC and offer an alternative model for the initial formation of vegetation.

**Grant:** 1R21DE014486-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** MCINERNEY, MARCIA F PHD  
**Title:** Analysis innate immune responses to oral pathogens  
**Institution:** UNIVERSITY OF TOLEDO TOLEDO, OH  
**Project Period:** 2002/08/05-2004/07/31

DESCRIPTION (provided by applicant): Diabetes is a risk factor for severe periodontal disease. Major pathogens associated with periodontitis are *Porphyromonas gingivalis* (*P. gingivalis*), *Bacteroides forsythus* (*B. forsythus*) and *Treponema denticola* (*T. denticola*), all Gram-negative anaerobes. Previous studies suggest that both innate and adaptive immunity are involved in protection against periodontal infection. Innate immune responses are the first line of defense against an infection. Innate immune system cells, such as macrophages, react to common microbial surface molecules through newly discovered receptors on the macrophage cell surface called Toll-like receptors (TLRs). Preliminary studies have found that lipopolysaccharide (LPS) derived from Gram-negative bacteria regulate the expression of several different TLRs in macrophages and trigger cytokine production and expression of co-stimulatory molecules in macrophages. These events are essential for macrophage activation and initiation of specific adaptive immune responses for generation of antigen specific cells. The purpose of this project is to study innate immunity in type 1 diabetes, in particular, the role of TLR in the initiation of host immune responses against oral pathogens in periodontal infection, using the well established non-obese diabetic (NOD) mouse model of type I diabetes. NOD macrophage responses to live bacteria and LPS isolated from *P. gingivalis*, *B. forsythus* and *T. denticola* in terms of cytokine production, co-stimulatory molecule expression, TLR mRNA levels and TLR signal transduction will be compared to NOR mice, a diabetes resistant control strain. Our hypothesis is that a defect in innate immunity in type 1 diabetes contributes to the susceptibility to periodontal infection since it should be the interaction between the TLR and the oral pathogen that initiates immune responses. These experiments will generate novel information on innate immune responses to oral pathogens in type 1 diabetes and may lead to development of therapeutic interventions to alleviate severe periodontitis in diabetes.

**Grant:** 1R21DE014565-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** PERIATHAMBY, ANTONY R PHD  
**Title:** Novel Bifunctional Molecules for Intraoral Drug Delivery  
**Institution:** MARQUETTE UNIVERSITY MILWAUKEE, WI  
**Project Period:** 2002/06/01-2004/05/31

**DESCRIPTION:** The goal of this innovative research project is to develop simple bifunctional molecules for intraoral delivery of antimicrobial agents. The bifunctional hybrid molecules will each be composed of a carrier sequence possessing high affinity for tooth and pellicle surfaces, and a natural antimicrobial peptide. These two sequences will be linked to each other with a biodegradable bond. With this linkage, antimicrobial sequences inherently linked to the carrier will be released efficiently from the tooth surface and in saliva through the oral physiological and microbial environment for a controlled and sustained release of the antimicrobial agent, thereby providing a novel and efficient method for intraoral drug delivery. This research project involves: 1) Synthesis of hybrid molecules by rationally selecting carrier sequences from salivary statherin, and antimicrobial sequences from bacteriocins and defensins; 2) Determination of toxicity of hybrid molecules to ensure that they are cytotoxic only to microbes; 3) Delineation of the effect of whole saliva on the stability of hybrid molecules; 4) Assessment of the adsorption and desorption characteristics of hybrid molecules onto hydroxyapatite surfaces; 5) Determination of the extent of adherence of *Candida albicans*, *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Streptococcus mutans*, *Streptococcus gordonii* and *Streptococcus sanguis* onto the hybrid adsorbed hydroxyapatite surfaces. This proposed research will identify new, safe, and affordable hybrid molecules for the prevention and treatment of plaque-related oral diseases. The hybrid molecules can be directly used as a topical rinse, or irrigant or they may be applied professionally to sub-gingival areas. The oral physiological and microbial environment will naturally induce the dissociation and the release of the antimicrobial peptide from the tooth surface into the site of oral infection. The hybrid molecules will serve as an efficient local drug delivery system and eliminate the discomfort and retention problems associated with the existing local delivery devices. The hybrid molecules will have a high potential for clinical and commercial application as anti-plaque agents.

**Grant:** 1R21DE014585-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** HUANG, GEORGE T.J DSC  
**Title:** Antimicrobial Gene Therapy  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2002/05/15-2004/04/30

**DESCRIPTION:** Increasing resistance to conventional antibiotics has focused attention on alternative anti-infectious therapies. Antimicrobial peptides are promising new agents that have a low susceptibility to microbial resistance mechanisms. Unlike conventional antibiotics, antimicrobial peptides are encoded by single genes and can be introduced into infected tissues by gene therapy approaches. The ultimate goal of this study is to introduce antimicrobial peptide genes into explanted human cells, and to use these augmented cells to enhance host defense mechanisms against infection. The immediate goal is to develop a model system, in which the human-Beta-defensin-2 (HBD-2) gene will be expressed in HBD-2 negative cells; its antimicrobial effect will be tested in vitro and in vivo. The HBD-2 gene was chosen because, unlike most known defensins, it does not require tissue-specific processing. A retrovirus carrying the HBD-2 gene has permitted successful transduction of mouse fibroblast NIH/3T3 cells to secrete functional antimicrobial peptides in vitro. Additionally, successful transduction of various other cell types to secrete HBD-2 has been accomplished. While antibacterial activity of HBD-2 in vitro has been clearly demonstrated, limited information is available regarding HBD-2 function in vivo. Using a novel approach, there are plans to test whether secreted HBD-2 is functional in vivo. The central hypothesis is that this antimicrobial gene therapy is effective in enhancing host innate immunity against infection. One short-term objective is to test whether the transduced cells will defend against infection in vivo using mouse models. The Specific Aims include: 1) to determine the temporal expression and the antimicrobial effects of HBD-2 in transplanted cells in vivo in a mouse model. This Aim will utilize SCID mice to grow HBD-2 expressing tumors which model will allow quantitative measurement of the antimicrobial effect of HBD-2 in vivo. 2) To establish a tissue-engineered wound healing model to test antimicrobial gene therapy in mice. This Aim will establish a model that simulates a clinical situation for testing the application of this antimicrobial gene therapy approach. Successful completion of these Aims will be the first step in establishing in vivo animal study models to explore the future applicability of antimicrobial gene therapy. The proposed approach is intended to provide potential benefit in clinical applications in three ways: 1) transplanted antimicrobial secreting cells can be used in wounded, engineered or infected tissues to prevent and/or fight against infections; 2) these cells may reduce the need for conventional antibiotics; and 3) these cells may provide long-term augmentation of the innate immune system for immunocompromised patients.

**Grant:** 1R21DE014896-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** KESAVALU, LAKSHMYA N DVM  
**Title:** n-3 Fatty Acid & Host Responses to Oral Infection  
**Institution:** UNIVERSITY OF KENTUCKY LEXINGTON, KY  
**Project Period:** 2002/09/15-2004/08/31

DESCRIPTION (provided by applicant): The major objectives of this R21 application are to explore the potential role of dietary n-3 polyunsaturated fatty acid (PUFA) regulation on molecular host responses to oral infection with Pg in vivo in an animal model. It is clear that host pro-inflammatory mediators provide a significant contribution to tissue destruction in chronic inflammation. Dietary n-3 fatty acid has been shown to modulate inflammatory responses via regulating lymphocyte proliferation, cytokine production, signal transduction, and gene expression in humans and rodents; providing a benefit by reducing inflammatory disorders, cardiovascular diseases, increasing anti tumorigenic effects on breast cancer, colon cancer, pancreatic neoplasms, and improving bacterial and autoimmune responses. Thus, we will initially focus on n-3 PUFA, which is the primary dietary lipid in "fish oil." We will determine its capability to modify host responses, affecting Pg pathogenesis, as a prototype periodontal pathogen. The experiments will utilize an in vivo rodent model of infection and alveolar bone resorption. Substantial evidence has established the contribution of host derived inflammatory cytokines in periodontal inflammation and disease that can lead to the alveolar bone loss and subsequent tooth loss, characteristic of periodontitis. To5knowledge, there are no in vivo investigations evaluating how n-3 PUFA regulates specific host-bacterial interactions in gingival tissues and alveolar bone resorption in periodontal disease. Two Specific Aims designed to test this hypothesis: 1) To examine the effect of dietary n-3 PUFA on gingival tissue expression of proinflammatory (TNFa, IL-1b,IL-6, lipid per oxidation, TBARS), anti-inflammatory (IL-10, TGF-b1, antioxidants) biomolecules, and T cell phenotypes (Th1, Th2) induced by Pg infection, and 2) To determine the effects of dietary n-3 fatty acid on Pg colonization and alveolar bone resorption. The long-range goal of this nutrition and oral infection project is to contribute to understanding the cellular and molecular mechanisms that enable dietary n-3 fatty acid to ameliorate tissue destructive aspects of periodontal pathogenesis. Positive findings could enable improved dietary strategies as adjuncts in the prevention of periodontal disease.

<b>Grant:</b>	1R21DE014997-01	
<b>Program Director:</b>	NOWJACK-RAYMER, RUTH	
<b>Principal Investigator:</b>	GALLAGHER, GRANT	BS
<b>Title:</b>	Immunogenetics and LAP susceptibility	
<b>Institution:</b>	UNIV OF MED/DENT NJ NEWARK	NEWARK, NJ
<b>Project Period:</b>	2002/09/30-2005/08/31	

DESCRIPTION (provided by applicant): Localized aggressive periodontitis (LAP) is a severe disease, which often leaves its victims with obvious, irreversible physical scarring. Susceptibility to this condition is up to fifteen times higher in African-American children than Caucasian children, but the reasons for this imbalance are completely unknown. Aggregation in families and this racial disparity indicate a strong genetic component to susceptibility but the molecular basis of this is presently undefined. This lack of understanding prevents the development of preventative measures and early treatment. This disease is known to have a major association with the bacterium *Actinobacillus actinomycetemcomitans*. Recent evidence including our preliminary data, indicate that genetic variation in those immune system genes used to respond to bacteria play a substantial role in the genetic susceptibility to LAP. The principal objective of this study is to identify genes which influence the occurrence of LAP in African-Americans. We propose to examine three independent but related groups of genes, each covering a complimentary aspect of human anti-bacterial defenses. First we shall examine opsonisation potential in neutrophils by genotyping the Fc-gamma genes. Second, we shall consider the genetic bias towards Th1 or Th2 responses in these patients and finally we shall use microarray analysis to define novel and hitherto unsuspected targets for future genetic and therapeutic studies. By integrating molecular immunogenetic techniques into a cross-sectional and longitudinal epidemiological study, it is our goal to ultimately develop a genetic susceptibility profile which can be used to identify children who are at risk from LAP. Additionally, the data harvested from these studies should allow earlier treatment of disease and enhance our understanding of the molecular mechanisms underlying the pathogenesis of LAP and other forms of periodontitis.

**Grant:** 1R21DE015077-01  
**Program Director:** NOKTA, MOSTAFA A  
**Principal Investigator:** PASTER, BRUCE J PHD  
**Title:** Oral microbial associations in HIV+ subjects  
**Institution:** FORSYTH INSTITUTE BOSTON, MA  
**Project Period:** 2002/09/01-2004/08/31

**DESCRIPTION** (provided by applicant): The broad objectives of this proposal are to examine the hypothesis that the more common periodontal infections in HIV+ subjects are caused by specific bacterial or fungal species, which are not necessarily the typical putative periodontal pathogens usually found in comparable periodontal infections in HIV negative subjects. Our preliminary data indicated that HIV+ subjects with necrotizing ulcerative periodontitis (NUP), a more severe periodontal disease unique to these individuals, indeed lacked many of the classical periodontal pathogens, but instead possessed "unusual" taxa, such as *Bulleidia extructa* and *Dialister pneumosintes*, and novel "uncultivable" phylotypes of the genera *Dialister*, *Peptostreptococcus*, *Selenomonas*, and members of the "uncultivable" phylum TM7. Our objectives are addressed in two Specific Aims. Aim 1 will identify the predominant cultivable and not-yet-cultivated bacteria and fungi that are associated with periodontal infections in HIV+ subjects, such as gingivitis, low/moderate and severe adult periodontitis, and Linear Gingival Erythema (LGE), another periodontal infection unique to HIV+ subjects. These studies will be performed by analyzing bacterial 16S rRNA and fungal 18S rRNA gene sequences of inserts in clones from plaque libraries. The libraries will be generated by PCR amplification of rRNA genes present in subgingival plaque followed by ligation of the amplicons into appropriate vectors and by transformation of *E. coli*. The sequence of each clone will be used to determine species identity, or if unknown, its phylogenetic position. It is anticipated that 25 to 50 additional new taxa will be identified. Aim 2 will test the hypothesis that specific bacterial and fungal species are associated with periodontal infections with the deterioration of immune status in HIV+ patients. In each periodontal disease category, 25 subjects with high viral loads and low CD4 levels and 25 subjects with low viral load and high CD4 levels will be analyzed. Comparisons of subgingival microbial and fungal populations will be made between these HIV+ groups vs. control groups of HIV negative subjects. Up to 200 predominant bacterial species will be assayed for each sample using Checkerboard hybridization, which allows for the simultaneous analysis of multiple samples with multiple probes. Up to 30 fungal species will be assayed for each sample in a second, separate checkerboard hybridization. It is likely that specific bacterial complexes (e.g., specific combinations of bacteria/fungi) are associated with these periodontal diseases and may change with the severity of HIV infection. The proposed studies may provide insight for the identity of putative pathogens in comparable periodontal infections in HIV negative individuals.



**Grant:** 1U01DE014955-01  
**Program Director:** KOUSVELARI, ELENI  
**Principal Investigator:** STAHL, DAVID A MS  
**Title:** DNA Microchips: Detecting Microbes in Oral Cavity Fluids  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/09/30-2006/06/30

DESCRIPTION (provided by applicant): The human oral cavity harbors complex microbial assemblages, which are associated with the manifestation or prevention of local and systemic disease. In addition, the oral cavity is one of the main ports of entry for potentially life-threatening agents released in the course of a bioterroristic attack. Conventional culture-based approaches for monitoring microbes in the oral cavity are not feasible since they are time-consuming and capture only a minor fraction of the oral microbiota. This project uses nucleic acid-based hybridization assays as an alternative to culture-based approaches. Specifically, target-specific and generic high-density DNA microchips will be used for the fast and reliable detection of microbial biomarkers in oral fluids. Two general formats of DNA microarrays will be used: (I) the OralChip, consisting of hierarchical sets of pro-and eukaryal ribosomal RNA-targeted oligonucleotide probes, and (II) the generic HexamerChip, containing all possible (4096) DNA hexamers. To further increase its diagnostic power, the OralChip will be expanded to include probes that detect the expression of genes involved in microbial community structure and virulence. Innovative statistical and mathematical tools will be applied for the integrated data analysis of both formats (i.e. artificial neural networks combined with conventional statistical approaches such as cluster analyses). This novel approach is expected to (i) provide population- and community-specific signature patterns of microbiota associated with oral microbial communities, ii) yield fast and unambiguous detection of biothreat-agents, (iii) determine the limitations (sensitivity, resolution) of the formats, and iv) reveal structural and functional attributes of the oral microbiota at high spatial and temporal resolution, specifically those associated with manifestation and prevention of local and systemic disease.

**Grant:** 2P01DK038030-16  
**Program Director:** SERRANO, JOSE  
**Principal Investigator:** HYLEMON, PHILLIP B PHD  
**Title:** LIVER/INTESTINAL METABOLISM OF BILE ACIDS/CHOLESTEROL  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 1986/12/01-2007/08/31

DESCRIPTION (provided by applicant): Background: Cholesterol and bile acids have been implicated in playing important roles in several major diseases of "Western Society" including: arteriosclerosis, cholesterol gallstone disease, cholestatic liver diseases, and colon cancer. The overall goal of this renewal application is aimed at a more detailed understanding of how the body regulates bile acid and cholesterol homeostasis, liver/intestinal physiology and determining if secondary bile acids are involved in the risk of cholesterol gallstone disease. The overall goal will be accomplished through the following specific aims: a) determine which cell signaling pathways are activated by bile acids in primary hepatocytes and which are important in regulating genes involved in cholesterol metabolism and phospholipid transport; b) determine if JNK-1 and JNK2 null mice are defective in cholesterol homeostatic mechanisms and if bile acid activated cell signaling pathways "cross-talk" with bile acid activated nuclear receptors e.g., FXR (Dent, Hylemon); c) characterize in detail the FTF/HNF-4 site in the sterol 12 $\alpha$ -hydroxylase (CYP8b1) promoter and elucidate the molecular mechanism by which FTF/SHP specifically regulates CYP8b1 transcription; d) characterize the molecular mechanism involved in the SREBP-2 mediated suppression of the CYP8B1 promoter; e) characterize the significance and physiological role SREB-2-mediated suppression of CYP8b1 (Gil); f) determine the role of steroidogenic acute regulatory (StAR) protein and other intracellular cholesterol transport proteins (SCP-2, MLN64) play in the regulation of bile acid synthesis in the liver; g) determine if StAR is expressed in the liver (Pandak,Gil); h) determine the 3 dimensional (3D) structure and catalytic mechanism of bile acid 7 $\alpha$  and 7 $\beta$ -dehydratase from *Clostridium scindens*; i) express, purify, and characterize a novel 3-oxo- $\Delta^4$ steroid oxidoreductase from *C. scindens*; j) clone, sequence, and analyze the bai operon from *Clostridium hylemonae* TN271; k) isolate, characterize, and identify cholic acid 7 $\alpha$ -dehydroxylating bacteria from cholesterol gallstone patients with high (>30%) deoxycholic acid and controls; and l) determine if gallstone patients are colonized by unique species of 7 $\alpha$ -dehydroxylating bacteria. (Hylemon, Heuman).

**Grant:** 1P01DK062041-01  
**Program Director:** MAY, MICHAEL K.  
**Principal Investigator:** MERCHANT, JUANITA L  
**Title:** Cellular Decisions of Differentiation in the GI Tract  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/09/15-2007/07/31

DESCRIPTION (provided by applicant): The Program Project Grant (PPG), "Cellular Decisions of Differentiation in the GI Tract" integrates the efforts of four investigators (two basic science and two clinical) from three Departments of the University of Michigan Medical School. The central goals of the work proposed in the PPG are: (a) To understand how epithelial cells in the upper gastrointestinal tract acquire their identity, both with respect to tissue identity (gastric vs. small intestine) and lineage identity (enteroendocrine cell vs. enterocyte or goblet cell) during ontogeny; (b) To investigate how the patterns of cellular differentiation in the acidsecreting epithelium of the stomach are normally controlled through specific intracellular pathways and how these pathways are altered by pathological insults (such insults can cause an alteration in identity of the gastric epithelium, such that it acquires a small intestinal phenotype). Subproject #1 examines the cis and trans factors that control identity in the intestinal epithelial cell, from a tissue-specific standpoint (stomach versus intestine), a regional standpoint (duodenum vs. distal intestine) and a differentiation standpoint (crypt vs. tip). In addition, in conjunction with Subprojects #2 and #4, the question of how gene regulation changes when stomach cells acquire intestinal identity (intestinal metaplasia) after pathological insult will be examined. Subproject #2 will trace the development of the enteroendocrine cell lineage within the intestine using cholecystikinin (CCK) as an early marker for this cell compartment. This work will utilize regulatory transgenes generated in Subproject #1. Subproject #3 will focus on how endogenous growth factors present in the stomach control the pattern of differentiation of the parietal cell. Subproject #4 examines the pathways through which the gastric epithelium responds to inflammation and/or bacterial overgrowth in the stomach through the generation of intestinal metaplasia, an alteration in gastric cell identity; the role of the pathways identified in Subproject #3 will be examined. Two Cores will assist the PPG investigators in the performance of Cell Biology techniques (Core A) and with Administration of the program (Core B). Both Cores will enhance the already strong interaction between these four investigators across Departmental lines. Overall, this PPG application will further our understanding of how cells make decisions of identity and differentiation in the stomach and intestine of the GI tract and will provide clues as to how this identity may be altered in pathological states.

**Grant:** 2R01DK012121-35  
**Program Director:** SECHI, SALVATORE  
**Principal Investigator:** AMES, GIOVANNA F PHD  
BIOCHEMISTRY:BIOCHEM  
RY-UNSPEC  
**Title:** Membranes and Active Transport of Amino Acids  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA  
OAKLAND  
**Project Period:** 1977/01/01-2005/06/30

DESCRIPTION (provided by applicant): ABC proteins or traffic ATPases are members of a large superfamily of translocators, both prokaryotic and eukaryotic. The eukaryotic members include the cystic fibrosis transmembrane regulator (CFTR), the P-glycoprotein of multidrug resistance (MDR), the gene responsible for adrenoleukodystrophy (ALD), the heterodimeric transporter associated with antigen processing (Tap1/Tap2), the transporter involved in Stargard macular dystrophy, and many others of medical importance. Among the prokaryotic members are bacterial periplasmic permeases, which have been extensively characterized. Considering the extensive homology between all of these proteins it is likely that there will also be strong similarities between eukaryotic and prokaryotic systems in their mechanism of action. Thus, prokaryotic permeases provide excellent models for achieving a general understanding of the activity and function of these translocators. The proposed work aims at understanding in detail the 3-dimensional structure of a model system: the histidine permease. We plan: 1). The spatial organization of the permease will be assessed by chemical cross-linking, NMR, and spin-labeling studies which will provide information on the three-dimensional structure by defining the nearest neighbor contact between the individual components. We will purify the individual cross-linked products, perform proteolytic digestion, HPLC separation of the peptides and mass spectrometry analysis. Comparison between patterns obtained from cross-linked products and from untreated complex will indicate the sites of contact. Experiments will also be performed on the permease in various stages of its activity cycle. 2). We will also try and obtain the crystal structure of the entire permease. If successful, this would provide the best description of the structure. 3). Crystal structures will be obtained of various mutants of the separated ATP-binding domains (HisP), which will provide information on which signals are needed to initiate ATP hydrolysis and on signaling mechanisms. Examination of these and other aspects of the histidine periplasmic permease will contribute to the understanding of general mechanistic questions for these transporters and will help unravel how their several sub-domains function. An understanding of the structure of these systems is essential for developing new approaches for investigating eukaryotic transporters. This knowledge can then be applied to help develop therapeutic tools for human diseases.

**Grant:** 2R01DK038955-14A1  
**Program Director:** NYBERG, LEROY M.  
**Principal Investigator:** KRIEGER, JOHN N  
**Title:** Urological Studies of Ideopathic Lower Tract Syndrome  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1991/08/01-2006/03/31

DESCRIPTION (provided by applicant): Prostatitis syndromes cause major morbidity with a 10 percent prevalence among adult men. This project focuses on the most common category, chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Long-term objectives are to determine the causes, consequences, and optimal therapy. Our working model is that bacterial infection is critical in many patients. Specific Aim #1. 16S rDNA evaluation, cloning, sequencing and phylogenetic studies. We will test the hypothesis that CP/CPPS patients have prostatic bacteria that distinguish them from controls. In pilot studies, patients with expressed prostatic secretion (EPS) inflammation (WBCs) were more likely to have bacterial ribosomal-encoding DNAs (16S rDNAs) than those without WBCs. We will clone and sequence 16S rDNAs from patients and controls. Sequences will be compared to available databases using BLAST searches and phylogeny software. These data will allow us to determine which bacteria are most specific to CP/CPPS, and, thus, which should be targeted in clinical trials. Specific Aim #2. Bacterial viability and clinical characteristics of CP/CPPS patients. We will test the hypothesis that bacterial viability correlates with the clinical severity of CP/CPPS. In pilot studies to evaluate bacterial viability, we developed quantitative assays for bacterial elongation messenger RNAs (tuf mRNAs) and documented that some CP/CPPS patients were tuf mRNA positive. We will compare clinical characteristics of patients with 16S rDNA and tuf mRNA, patients with 16S rDNAs but no tuf mRNA, and those without 16S rDNA or tuf mRNA. The tuf mRNA studies will be correlated with improved cultures of the same specimens. This study will provide insights into the potential value of antimicrobial therapy and identify characteristics that distinguish patients most likely to respond. Specific Aim #3. Comparison of prostatic bacteria with EPS and seminal fluid (SFA) bacteria. We will test the hypotheses that CP/CPPS patients with prostatic bacteria have similar bacteria in their EPS and/or seminal fluid (SFA) and, further, that these bacteria differ from the bacteria in EPS and/or SFA of controls. Our preliminary studies identified the most common bacteria in CP/CPPS patients' prostatic parenchyma. To show that CP/CPPS patients have similar bacteria in their SFA and/or EPS, we will determine homology of 16S rDNAs in EPS, SFA, and prostate biopsy material from individual patients. To show that these bacteria differ from the bacteria in controls, we will compare 16S rDNAs in SFA and EPS of CP/CPPS with controls. These studies will determine if EPS or SFA can be used to identify prostatic bacteria and may result in clinical methods for non-invasive diagnosis of prostatic infection.

**Grant:** 2R01DK045496-10  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** SEARS, CYNTHIA L MD  
**Title:** Physiology and Cloning of B. Fragilis Enterotoxin  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1993/08/20-2006/07/31

DESCRIPTION (provided by applicant): *Bacteroides fragillis* are the leading causes of anaerobic bacteremia and intraabdominal abscesses. Certain strains of *B. fragilis* termed enterotoxigenic *B. fragillis* (ETBF) are linked epidemiologically to diarrheal illnesses in animals, children and adults. The only recognized virulence factor of ETBF is a secreted 20 kDa zinc-dependent metalloprotease toxin termed the *B. fragillis* toxin (BFT). BFT has sequence homology to eukaryotic matrix metalloproteases (MMPs) of the metzincin family. By cell-surface proteolysis, MMPs regulate eukaryotic cell signal transduction and function. Our data reveal that BFT treatment of intestinal epithelial cells in vitro results acutely in cleavage of the extracellular domain of E-cadherin (the key intercellular adhesion protein of the intestinal epithelial cell zonula adherens) and stimulation of several signal transduction pathways (Beta-catenin, tyrosine kinase, nuclear factor-KB and mitogen-activated kinases) yielding cellular cytoskeletal, physiologic and nuclear activation sequelae. Thus, we postulate BET is a prokaryotic mimic of the eukaryotic MMPs. Our preliminary data also suggest that BET binds to a sialic acid-containing glycoprotein. Based on our data, we postulate that BFT binds to a specific cell receptor, possibly a receptor tyrosine kinase. Activation of a tyrosine kinase(s) contributes to E-cadherin cleavage and induces expression and secretion of the proinflammatory chemokine, interleukin-8, by intestinal epithelial cells. E-cadherin cleavage results in: 1) reorganization of the apical cytoskeleton of intestinal epithelial cells leading to reduced barrier function and chloride secretion; and 2) T cell factor (TCF)-dependent Beta-catenin nuclear signaling resulting in c-Myc induction and cellular proliferation. The precise cellular substrate for BET is not clear but is postulated to be E-cadherin or the cellular receptor for BFT. Our long range goal is to define in detail the cellular mechanism of action of BFT. We now propose to identify the very early steps in the mechanism of action of BET and to define the functional domains of the BET protein. Our specific aims are: 1) to determine the impact of the intercellular adhesion protein, E-cadherin, in mediating the mechanism of action of BET. The E-cadherin cleavage site will be identified and mutated such that it is resistant to BFT-initiated proteolysis. The BET biologic activities attributable to E-cadherin cleavage will be unambiguously identified; 2) to investigate the functional domains of BFT including the catalytic domain, the minimal protein domain yielding BET biologic activity and the function of cysteines in the proprotein toxin domain; and 3) to establish that BFT binds specifically to a cellular receptor and to identify the BFT receptor. These studies will advance our understanding of the mechanism of action of a novel and potent bacterial toxin and may provide a molecular rationale for human investigations to further define the morbidity of ETBF colonization and disease.

**Grant:** 2R01DK051406-06

**Program Director:** MULLINS, CHRISTOPHER V.

**Principal Investigator:** HULTGREN, SCOTT J PHD MICROBIOLOGY  
MOLECULAR BIOLOGY

**Title:** Molecular Basis of E. coli Adhesins in Bladder Disorders

**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO

**Project Period:** 1997/01/01-2006/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): Urinary tract infections (UTIs) are common infections that affect a large proportion of the world population and account for significant morbidity and medical expenditures. These infections are most commonly caused by *Escherichia coli* (*E. coli*). A long-term goal of this proposal is to understand the processes by which *E. coli* causes acute, recurrent and chronic UTIs and the sequelae of these infections. An integrated approach will be used that blends a powerful bacterial genetic system, a mouse UTI model, and x-ray crystallography with high-resolution electron microscopy (EM), protein chemistry, carbohydrate chemistry, and tissue culture systems in order to reveal the cellular, molecular, and structural basis for the pathogenesis of these infections. The FimH adhesin present at the tip of type 1 pili has been shown in animal models to mediate binding to the uroplakin-coated luminal surface of the bladder. The uroplakin receptor complexes recognized by the FimH adhesin will be cloned and used to investigate the consequences of FimH-uroplakin interactions. Also, the three dimensional structure of the FimH adhesin will be used to design a panel of site directed mutations to delineate the mannose binding pocket of the FimH adhesin and the structural basis of bacterial colonization of the urinary tract. The adaptive responses to bladder infections and the activation of signals that lead to the release of cytokines and recruitment of neutrophils will be dissected in detail. FimH-mediated attachment to the bladder epithelial cells activates a cascade of innate defenses that leads to rapid exfoliation and proliferation of underlying epithelial cells. The molecular basis of exfoliation will be investigated and its role in protecting the bladder from infection will be studied. The molecular mechanisms by which uropathogenic *E. coli* are able to invade bladder epithelial cells and evade the host response will be elucidated. Uropathogenic *E. coli* replicate intracellularly and form "bacterial factories" in the luminal superficial facet cells of the bladder. The virulence factors required for this process will be identified and studied. Finally, the fluxing of *E. coli* out of the facet cells and colonization of underlying tissue will be investigated as a mechanism to cause persistent and recurrent infections. These studies will contribute to the development of adhesin-based vaccines to treat and prevent urinary tract infections and their sequelae.

**Grant:** 2R01DK055812-04  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** COMINELLI, FABIO MD  
**Title:** Mechanisms of Experimental Crohn's Disease  
**Institution:** UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA  
CHARLOTTESVILLE  
**Project Period:** 1999/07/01-2007/06/30

DESCRIPTION (provided by applicant): Crohn's disease (CD) is a debilitating condition of unknown etiology that is poorly responsive to currently available treatments. A working hypothesis suggests that CD may represent a dysregulated immune response to antigens derived from normal intestinal bacteria in a genetically predisposed host. Our studies will take advantage of a new strain of mice referred to as SAMP1/Yit/FC that develops enteritis spontaneously without genetic or immunologic manipulations. Preliminary studies indicate that these mice develop disease only when colonized with normal murine intestinal flora and not when derived under germfree conditions. The central hypothesis of this proposal is that in SAMP1/Yit/FC mice, normal intestinal bacteria are required for the development of ileitis through the induction of antigen-specific immune responses in the gut, and that proper manipulation of the bacterial flora may result in disease amelioration. The overall objective of this proposal is to investigate, in a mechanistic fashion, the role of the bacterial flora in experimental CD. In order to achieve our goals we will: 1) Determine the effects of antibiotic/probiotic administration on the severity of ileitis. The effects of antibiotic administration, as well as that of well-characterized probiotics will be investigated. In addition, the ability of antibiotic/probiotic treatment to maintain remission following initial treatment with anti-TNF or prednisone will be studied; 2) Characterize the composition of the bacterial flora as well as its functional effects on T cell activation. State-of-the-art techniques, including 16S rRNA PCR will be used to characterize the bacterial flora colonizing the ileum versus the colon of SAMP1/Yit/FC mice. In addition, a series of in vitro T-cell activation studies as well as adoptive transfer experiments following stimulation with indigenous flora will be used to attempt to define the pathogenesis of ileitis in these mice; 3) Determine the effects of germ-free conditions and the role of specific bacterial species on chronic intestinal inflammation. SAMP1/Yit/FC mice will be re-derived under specific germ-free conditions and specific bacterial species will be re-introduced to determine their ability to induce chronic ileitis in germ-free mice. In addition, adoptive transfer experiments will be performed to define whether the primary abnormality of SAMP1/Yit/FC mice occurs in the T cells or is caused by specific bacterial antigens.



**Grant:** 1R01DK060049-01A1  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** PODOLSKY, DANIEL K  
**Title:** Toll-like Receptors and the Intestinal Epithelium  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2002/04/01-2007/03/31

DESCRIPTION (provided by applicant): The intestinal mucosa exists in a functional equilibrium with the complex luminal milieu, which is dominated by a wide variety of commensal microbial species. Despite the continuous confrontation with bacterial products the normal mucosa exists in a state of minimal immune activation but must promptly mount an effective defense typically dependent on activation of inflammatory pathway when infection with a pathogen occurs. The overall goal of the present proposal is to define the mechanisms through which the epithelium interfaces with normal and pathogenic luminal bacteria and the processes determining the functional outcome of those interactions whether normal homeostasis or active inflammation. These studies are based on the hypothesis that a recently identified family of pattern recognition receptor designated toll-like receptors (TLRs) found in preliminary studies to be present on the apical surface of intestinal epithelial cells allow the mucosa to sample the bacterial milieu and play a determining role in the functional outcome of that interaction. We also hypothesize that functional dysregulation of the TLR mediated responses eventuate in chronic intestinal inflammation. These preliminary studies have suggested that TLR4 which binds the ubiquitous bacterial cell wall product lipopolysaccharide (LPS) and TLR3 which binds other cell wall products may be especially pivotal as suggested by the finding of distinctive alterations in expression in human inflammatory bowel disease. These hypotheses will be evaluated through studies designed to address three specific aims: (I) To define the signaling pathways activated in intestinal epithelial cells by different TLRs following binding of cognate ligands, (II) To delineate the functional response of intestinal epithelial cells mediated through activation of TLRs, and (III) To determine the expression and functions of TLRs in inflammatory bowel disease in murine models and human IBD. These studies will encompass detailed assessment of the function of TLRs in model intestinal epithelial cells in vitro and both human tissues and novel murine models in vivo. Collectively these studies should provide new insights into the role of the TLRs, key elements of the innate immune system, in modulating the dynamic balance between controlled surveillance and appropriate response to mucosal challenge by luminal flora.

**Grant:** 1R01DK061707-01  
**Program Director:** KARP, ROBERT W  
**Principal Investigator:** NUNEZ, GABRIEL MD  
**Title:** Nod2: A Susceptibility Gene for Crohn's Disease  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/04/08-2007/03/31

The idiopathic inflammatory bowel diseases (IBD) which includes Crohn's disease and ulcerative colitis are chronic disorders of the gastrointestinal tract of unknown etiology with a combined prevalence of about 150-200 cases per 100,000 in western countries. Although the etiology of IBD is unknown, a large body of evidence suggest that these diseases are multifactorial and likely caused by an abnormal inflammatory response directed against luminal and/or enteric microflora in a genetically susceptible host. However, the genetic basis for this abnormal inflammatory response to enteric bacteria is unknown. Genome-wide searches for IBD-susceptibility genes have resulted in the identification of several loci harboring potential predisposing genes for Crohn's disease. Of these, linkage to the pericentromeric region of chromosome 16 (IBD1 locus) has been replicated by several independent studies to confer susceptibility to disease. We have identified Nod2, a gene that encodes a protein with homology to plant disease resistance gene products, that is located in the peak region of linkage disequilibrium on chromosome 16. We have found that a frameshift mutation and genetic variants of Nod2 are highly associated with susceptibility to Crohn's disease by genetic analysis in multi-case disease families and case-control studies. Nod2 is expressed in monocytes and activates NF- $\kappa$ B. Significantly, wild-type Nod2 confers responsiveness to bacterial lipopolysaccharides and this activity is deficient in mutant-Nod2 associated with Crohn's disease. These observations suggest a link between an innate immunity pathway controlled : byNod2 and susceptibility to Crohn's disease. Our overall hypothesis is that Nod2 recognizes lipopolysaccharidesin the cytosol and activates a NF- $\kappa$ B signaling pathway in the host cell that protects the host against entericbacteria. Our preliminary results suggest a model in which deficiency in the Nod2 pathway leads to an abnormal T cell-mediated response to enteric bacteria and tissue destruction. We propose three Specific Aims to explore our hypothesis: (i) Determine the sequence of Nod2 that mediates functional activity and - recognition of bacterial LPS. The analyses will include study of Nod2 variants associated with Crohn's disease and systematic mutagenesis of Nod2; (ii) Determine the structure of LPS recognized by Nod2 and (iii) Characterize mice deficient in Nod2 to determine its role in the response to luminal and pathogenic enteric bacteria. The proposed studies should improve our understanding of the role of Nod2 in innate immunity and provide-important insight into the link between genetic variation in Nod2 and susceptibility to Crohn's disease. The studies may lead to novel therapeutic approaches for Crohn's disease.

**Grant:** 1R01DK063092-01  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** GRAHAM, DAVID Y.  
**Title:** The Role of Mycobacteria in Crohn's Disease  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2002/05/01-2006/04/30

DESCRIPTION (provided by applicant): Crohn's disease is an idiopathic non-caseating granulomatous disease. One of the proposed etiologies is infection with *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*), the causative agent of Crohn's-like disease in ruminants (Johne's disease). Recent evidences to support *M. paratuberculosis* infection in Crohn's disease include: 1) its isolation from Crohn's disease tissues and breast milk by culture, 2) its identification in tissues by PCR assays, 3) its detection as cell wall deficient forms in tissues by in situ hybridization, 4) the long term remission (possibly cure) in an increasing number of Crohn's patients by using anti-mycobacterial therapies, and 5) by an association with the *M. paratuberculosis* antigens p35, p36 and the 32k mycobacterial associated antigen termed HupB protein. These data suggest a causal role for mycobacteria in at least a proportion of patients with Crohn's disease. Identification of the subgroup of Crohn's disease patients infected with *M. paratuberculosis* has been hampered due to the lack of a simple and specific serodiagnostic test to identify those who would be candidates for anti-mycobacterial therapy. The long-range objective of this proposal is to confirm *M. paratuberculosis* p35/ p36 antigens as serologic markers and to test whether there are specific clinical/pathologic stratification(s) that correlate with their presence. We will assess the presence of *M. paratuberculosis* infection in Crohn's disease patients by serologic testing of sera from patients and controls and in situ hybridization for the detection of the cell wall-deficient form of *M. paratuberculosis* in involved diseased tissues. We will also use the laser capture microdissection technique to test whether *M. paratuberculosis* are present in granulomas of Crohn's disease patients. The results from serology and molecular studies will be compared with the clinical/pathological information and demographic and epidemiologic data gathered about each patient as well as with outcome of anti-mycobacterial therapy. The results of this study should either confirm or refute the proposed etiologic association of *M. paratuberculosis* and Crohn's disease as well as the identification of patients with Crohn's disease and *M. paratuberculosis*.

**Grant:** 1R03DK060656-01  
**Program Director:** EVERHART, JAMES  
**Principal Investigator:** LEW, EDWARD A MD  
**Title:** Aspirin, Helicobacter Pylori, and Peptic Ulcer Disease  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 2002/02/01-2004/01/31

Aspirin and other non-steroidal anti-inflammatory drugs are widely used to treat pain and inflammation, and at low doses, aspirin is also increasingly being used for cardiovascular prophylaxis. However, these drugs have substantial gastrointestinal toxicity and a significant number of patients develop peptic ulcers and GI bleeding. Although infection with *Helicobacter pylori* is another major risk factor for ulcers, the relationships between aspirin and *H. pylori* in the development of ulcers remain highly controversial. It is unclear, for example, whether there is an additive or synergistic interaction between these factors in conferring ulcer risk such that aspirin interacts with *H. pylori* to increase ulcer complications. Aspirin impairs mucosal protective mechanisms by decreasing prostaglandin production, whereas *H. pylori* promotes mucosal injury through cytokines and inflammation to form ulcers. Past studies have provided conflicting data on the ulcer risks associated with both factors but they have been limited by recall bias of aspirin use, selection bias, and small sample sizes with short follow-up. The primary goals of the proposed research are to determine the risk of peptic ulcers associated with the joint effects of low dose aspirin and *H. pylori* infection, the ulcer risk associated with low dose aspirin and a specific virulent strain of *H. pylori*, known as cagA+ *H. pylori*, and the risk of GI bleeding associated with low dose aspirin and *H. pylori* (especially cagA+ *H. pylori* strains) as compared to those without infection. We will have 80% power to detect a difference of 1.56 in the odds ratio, when comparing the association of aspirin use and ulcer formation in *H. pylori* positive and negative subjects. As the US population grows older, the chronic use of aspirin for cardiovascular prophylaxis and the subsequent development of ulcers are likely to increase, involving health care costs. The proposed study will provide important information to make an informed decision about aspirin related GI complications and whether *H. pylori* infected patients are at risk for ulcers and GI bleeding while on aspirin. These results may help identify high-risk patients and lead to strategies that will reduce ulcer complications among aspirin users.

**Grant:** 1R15DK058128-01A1  
**Program Director:** MCKEON, CATHERINE  
**Principal Investigator:** YU, HONGWEI PHD  
**Title:** Aerosol Infection Mouse Model for Cystic Fibrosis  
**Institution:** MARSHALL UNIVERSITY HUNTINGTON, WV  
**Project Period:** 2002/04/01-2004/03/31

Chronic respiratory infections with *Pseudomonas aeruginosa* are the primary causes of high morbidity and mortality in cystic fibrosis (CF). We have recently developed a unique pulmonary infection mouse model that depends on the artificially generated *P. aeruginosa* aerosol to cause a uniform whole-lung infection in mice. The focus of this revised proposal is to test a group of 90 clinical CF isolates of *P. aeruginosa* for innate lung clearance, cytokine profiles and histopathology in this aerosol infection model. The hypothesis to be tested here is that the hypervariable chromosomal restriction fragment length polymorphisms (RFLPs) of the clinical CF sputum isolates may contribute to i) variations in bacterial respiratory colonization capacity, ii) altered levels of cytokine production by the host, and iii) the different outcomes of lung pathology. This is based on our following recent observations. First, we have applied the technique of pulsed field gel electrophoresis (PFGE) to analyze a collection of 90 clinical CF isolates for their genomic profiles. We have established a database composed of 75 unique Spe-I restriction digest PFGE profiles. Out of 90 strains tested, we identified one isolate CF32 that had identical Spe-I, Xba-I and Dpn-I digest PFGE patterns as *P. Aeruginosa* PAO1, a standard reference strain of a wound origin. Secondly, we passed PAO1 and 3 other CF isolates including the PAO-1 like CF isolate through the aerosol infection system to test for the pulmonary clearance and production of tumor necrosis factor (TNF)- $\alpha$ . PAO1 and CF32 showed a similar pattern of lung clearance and TNF- $\alpha$  induction in the C57BL/6J and BALB/cJ mice. However, the other 2 CF isolates were more resistant to the clearance by the BALB/cJ mice. One isolate (CF45) caused a significant induction of TNF- $\alpha$  by the murine lungs. These results indicate that the genomes of the CF isolates are highly diversified, and the genomic diversity may affect their intrinsic biological properties. More importantly, it's feasible to use the aerosol apparatus to assess the remaining CF isolates for their virulence properties. The future directions that this project may lead to include i) investigations of the novel *Pseudomonas* genes induced due to lung colonizations; ii) exploration of the novel DNA fragments missing in the PAO1 genome but present in a subset of the CF isolates; iii) DNA immunization and testing for protection, and iv) evaluation of some selected CF isolates in the aerosol mouse model. By achieving the research objective of this Academic Research Enhancement Award (AREA) that is to establish and infection database for the CF isolates in the aerosol model, we will have an essential base of knowledge from which to prepare a future R01 application to investigate the novel virulence mechanisms associated with the clinical CF isolates of *P. aeruginosa*.

**Grant:** 1R21DK060847-01  
**Program Director:** RASOOLY, REBEKAH S.  
**Principal Investigator:** TURNER, GEORGE J  
**Title:** Overexpression of angiotensin receptors in Halobacteria  
**Institution:** UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL  
**Project Period:** 2002/04/01-2004/03/31

All cells sense their environment through membrane proteins (e.g. receptors and ion channels) and regulate their physiological responses accordingly. Angiotensin receptors are involved in an array of physiological processes including fetal kidney development, electrolyte homeostasis, and blood pressure control. Angiotensin receptor activation has also been linked to cellular phenotypic changes and cell growth, gene expression of hormones, growth factors, cytokines, and activation of multiple intracellular signaling cascades. Numerous studies have revealed that the pathophysiology of certain cardiovascular and renal diseases in humans is a result of aberrant activation of angiotensin receptors. A detailed molecular understanding of how receptors perceive and transfer information across cellular membranes is lacking due to limited knowledge of membrane protein structures and forces regulating their function. A goal of our work is to develop a useful expression system for over-expressing channels, transporters, and receptors critical for renal homeostasis. This system will produce the amounts of protein required to determine the structure of receptors. Our approach is to convert a naturally occurring membrane protein expression system to general usage. Toward that goal we have engineered a series of prototype expression vectors that will drive expression of the angiotensin receptor gene by elements known to direct the high levels of transcription and translation of the bacterio-opsin gene in the Archaeon *H. salinarum*. We will also use this new expression system to express a portion of the angiotensin receptor as a fusion to BR and use this fusion protein as an antigen allowing production of angiotensin receptor antibodies. This work is feasible and represents new methodology that will be made generally available for the study of receptors, channels, and transporters.

**Grant:** 1R21DK063603-01  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** TCHOU-WONG, KAM-MENG M PHD ENVIRONMENTAL  
HEALTH  
**Title:** Role of H. Pylori Infection in Esophageal Adenocarcinoma  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY  
MEDICINE  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): The prevalence of *Helicobacter pylori* colonisation in populations in developed country has been declining. The decreasing prevalence of cagA+ *Helicobacter pylori* may be associated with the rising incidence of esophageal adenocarcinomas in industrialized countries. Colonization with cagA+ strains has been shown to be inversely associated with reflux esophagitis and Barrett's esophagus. A lower prevalence of cagA+ *Helicobacter pylori* has been observed in patient with gastroesophageal reflux disease (GERD) which results from acid exposure to the esophagus. One explanation for the negative association between colonization with *Helicobacter pylori* and GERD is the effect of *Helicobacter pylori* on acid production. Eradication of *Helicobacter pylori* has led to the development of GERD in a proportion of treated patients. These clinical evidence has led to the hypothesis that *Helicobacter pylori* could play a protective role in the development of GERD, especially reflux esophagitis. Experiments proposed in the following specific aims will test the hypothesis that *Helicobacter pylori*, especially the cagA+ strains, may protect against GERD, Barrett's esophagus and esophageal adenocarcinoma. The specific aims are as follows: 1. To study the effects of gastric colonization of cagA+ and cagA- strains of *Helicobacter pylori* on host inflammatory responses in rats and mice. 2. To determine the effects of *Helicobacter pylori* infection in reflux esophagitis, Barrett's esophagus and esophageal adenocarcinoma in a surgical reflux model in rats. 3. To determine the effects of *Helicobacter pylori* infection in esophagitis, Barrett's esophagus and esophageal adenocarcinoma in a surgical reflux model in wild-type and p53 knockout mice. The proposed studies aim to ascertain the role of *Helicobacter pylori* colonization in the development of reflux esophagitis, Barrett's esophagus and its associated adenocarcinoma in rodent models. This proposal utilizes the innovative surgical models of GERD, BE and EAC for studying the protective role of *Helicobacter pylori* against reflux complications.

**Grant:** 1R01EB000720-01  
**Program Director:** MOY, PETER  
**Principal Investigator:** SHIH, WAN Y PHD  
**Title:** Quantitative Array Piezoelectric Microcantilever Sensors  
**Institution:** DREXEL UNIVERSITY PHILADELPHIA, PA  
**Project Period:** 2002/09/30-2005/08/31

**DESCRIPTION** (provided by applicant): **Objective:** The goal of proposed research is to fabricate and use highly piezoelectric microcantilever arrays for ultrasensitive, in-situ, rapid, simultaneous multiple analyte quantification in small sample volumes using electrical means with unprecedented sensitivity (10<sup>-15</sup> g). The proposed piezoelectric microcantilevers with antibodies specific to the target antigen immobilized at the cantilever tip will measure the presence of proteins or pathogens with femtogram (smaller than the mass of a single cell) sensitivity. This represents the ability to detect a single cell or about 1000 molecules in a small volume. We will demonstrate the application with (1) transient protein expression of a recombinant protein, alkaline phosphatase (SEAP) in Chinese Hamster Ovary cells (CHO) during growth in a bioreactor, and (2) early detection of streptococcus at ultra low concentrations, 100 - 10,000 number/ml. **Approaches:** The device consists of a highly piezoelectric lead magnesium niobate-lead titanate solid solution (PMN-PT) cantilever smaller than 50  $\mu$ m in length coupled to antibody immobilized at the cantilever tip. Binding of target antigens is detected by monitoring the resonance frequency shift. Because of the small sizes, the cantilevers will be capable of detecting a single cell or some 1000 molecules in a small volume. The resonance frequency shift transient will be used to quantify the amount of antigens present in a small volume. In the model CHO bioreactor, the SEAP expression quantification is used as an example to illustrate the proposed methodology. For pathogen detection, microcantilevers developed under this program have the potential to detect streptococcus at a concentration of 100 to 10,000 bacterial/ml, significantly enhancing detection capability. Experiments will be designed to explore lower level of detection in the range of 100 to 10,000 cells/ml. Because the proposed piezoelectric cantilever sensors use electrical signal for actuation and detection, the sensor and all necessary electronics can be organized in a compact form and easily usable in such broad ranging applications such as early disease detection and genomics-inspired proteomics. **Results:** It is anticipated that as a result of the proposed study, ultra sensitive, rapid, specific, multiple analyte quantification in ultra low concentrations and small sample volumes will be achieved using arrays of highly piezoelectric PMN-PT micro cantilevers of less than 50  $\mu$ m in length with better than 10<sup>-15</sup> g/Hz sensitivity coupled with antibodies specific to the target antigen immobilized at the cantilever tip with simple electrical means, which has wide ranging applications such early disease detection and genomics-inspired proteomics.



**Grant:** 1R21EB000979-01  
**Program Director:** KELLEY, CHRISTINE  
**Principal Investigator:** SIMON, SANFORD M PHD  
**Title:** Large Scale Chemical Screen Against Pathogenic Bacteria  
**Institution:** ROCKEFELLER UNIVERSITY NEW YORK, NY  
**Project Period:** 2002/09/15-2004/08/31

DESCRIPTION (provided by applicant): Gram negative bacteria are surrounded by two membranes that shield them from the outside world. The viability of the bacteria depends upon the integrity of this membrane barrier. The pathogenicity of many gram-negative bacteria such as Yersinia, Salmonella, Shigella, Erwinia and pathogenic Escherichia coli depend upon their ability to export their toxins across these membranes without compromising the integrity of the membrane barrier. Considerable homology exists between the export pathways used for export of filamentous phage, type II secretion for the export of toxins or degradative enzymes into the extracellular milieu and type III secretion, in which proteaceous toxins are secreted and injected directly into the cytosol of eukaryotic host cells, causing cytotoxicity. The export pathway for filamentous phage f1 forms a transmembrane aqueous channel ) through which the phage traverse during biogenesis. The opening of this export channel is tightly regulated. The channel is normally closed and only opens to allow the extrusion of filamentous phage (or in the case of type II or type III secretion, the export of toxin). If this channel opens inappropriately, the viability of the host bacteria is significantly compromised . The permeability of this channel can be studied with a colorimetric assay that is compatible with a high-throughput screen for agents that could open these channels. These export channels only exist in pathogenic bacteria. They are encoded on the pathogenicity islands and are not part of the host bacterial genome. This is an application for a large-scale chemical screen for agents that affect the gating of these channels and open them. This could generate an agent that would selectively target a particular pathogenic bacteria and leave the rest of bacterial flora intact. Each of the export proteins from the gram-negative pathogens will be cloned and expressed in an E. coli system that has been optimized for the colorimetric detection of channels that are open. These E. coli will then be screened with a chemical library to detect agents that open these channels.

**Grant:** 1R21EB000980-01  
**Program Director:** MOY, PETER  
**Principal Investigator:** DALY, DON SIMONE PHD  
**Title:** Microbial Fingerprinting Chip and Automated Analysis  
**Institution:** BATTELLE PACIFIC NORTHWEST RICHLAND, WA  
LABORATORIES  
**Project Period:** 2002/09/30-2004/08/31

DESCRIPTION (provided by applicant): DNA fingerprinting provides the capability to identify and genetically discriminate between closely related strains of microorganisms, which is required for forensic identification and tracking of disseminated bio-agents or pathogens (e.g., *Bacillus anthracis*). However, the development and application of advanced diagnostic techniques for the identification and characterization of microorganisms continues to be limited by the absence of instrumentation and methods that are (1) capable of discriminating between closely related microorganisms, (2) rapid, easy-to-use, and conducive to automation, (3) capable of producing statistically rigorous data with relative ease, and (4) highly reproducible. The continued inability of forensic scientists to locate the geological or institutional origin of *B. anthracis* released into the U.S. Postal Service highlights the continued need for a rapid, automated, objective, and high-resolution diagnostic system for microorganisms. The objective of this project is therefore to demonstrate a universal DNA fingerprinting microarray and statistically based data analysis algorithms for the forensic identification and diagnosis of *B. anthracis* and/or near-neighbors within the *B. cereus* complex. The universal DNA fingerprinting microarray proposed here utilizes the power of "genome scanning" and "sequencing by hybridization" to interrogate a larger proportion of the genome than can be accessed with current PCR- or gel-based methods. In contrast to PCR or gene-probe methods, the universal fingerprinting microarray may further be capable of identifying "emerging" or otherwise "unknown" microorganisms for which specific gene sequences are not available or known. Because the method and DNA chip is "universal", it may be applicable for the identification of other microorganism, as demonstrated in our preliminary studies with *Salmonella*, *Xanthomonas* and *E. coli* pathogens.

**Grant:** 1R21EB000981-01  
**Program Director:** KORTE, BRENDA  
**Principal Investigator:** LAKOWICZ, JOSEPH R  
**Title:** Biohazard Detection Using Metal-Enhanced Fluorescence  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD  
**Project Period:** 2002/09/10-2004/08/31

DESCRIPTION (provided by applicant): Recent results from this laboratory have shown that the spectral properties of fluorescent probes can be favorably enhanced by interactions with metallic particles. We propose to use these interactions to develop high sensitivity detection of bioterrorism-related pathogens. The overall goal is to demonstrate single molecule or low copy number detection of nucleic acid sequences specific for such organisms. To accomplish these goals we propose: Specific Aim 1. Develop and test specificity of microarrays for selected *Y. pestis* and *Y. pseudotuberculosis* genes. We will select genes from the known genomic sequence of *Yersinia pestis*. Some genes will be specific to the virulence plasmids and others to detect both *Y. pestis* and the less virulent *Y. pseudotuberculosis*. The specificity will be determined against DNA from other species and strains of *Yersinia*. Specific Aim 2. Optimization of the fluorophore-metallic particle detection methodology for use with pathogen-specific oligomers. We will optimize the fluorophore-metallic surface geometries to obtain the maximal number of photons per fluorophore. This will include: examination of silver island films and colloids to determine the optimal size and shape for enhanced emission; evaluation of the optimal distance from the fluorophore to the metallic surface for maximal emission; and evaluation of the use of FRET for DNA assays. Specific Aims 1 and 2 will proceed concurrently. Specific Aim 3. Determine the sensitivity and specificity of the metallic-surface enhanced assays for *Y. pestis*. The sensitivity of the assays will be determined by assays based on serial dilutions. The specificity will be determined against DNA from other species and strains of *Yersinia*.

**Grant:** 1R21EB000982-01  
**Program Director:** KORTE, BRENDA  
**Principal Investigator:** BASHIR, RASHID PHD  
**Title:** Rapid Determination of Viability of Anthrax Spores  
**Institution:** PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN  
**Project Period:** 2002/09/25-2004/08/31

DESCRIPTION (provided by applicant): Micro- and nano-systems technology has found increasing use in a wide variety of biomedical applications, including detection and characterization of biological entities. The devices used for such applications are broadly referred to as 'bio-chips' and for example in the case of DNA detection, have even been commercialized. One area of research that has become increasingly important but not very well studied is the handling, manipulation, and characterization of very few cells and micro-organisms using biomedical micro-electro-mechanical-systems technology (BioMEMS). The detection of very low cell concentrations from samples of bodily fluids, tissue samples, soil, water, and food is a challenge that has not been fully realized. The goal of such an effort should be to handle, detect, and characterize a single cell or microorganism, and micro-devices are ideally suited for such studies. In addition, reducing the time-to-result to be able to perform "point-of-use" analysis is also very important. Such endeavors can not only yield very important scientific results but can also be used immediately in practical diagnostic applications in the health and food industry and in biological warfare and hazard prevention systems. This project brings together interdisciplinary researchers from the fields of micro/nano-systems technology and microbiology with the knowledge of *Bacillus anthracis* (anthrax) to further the state-of-the-art in micro-scale detection and identification of *Bacillus anthracis*. The knowledge developed herein will also apply to other microorganisms. The PIs have developed novel technologies that serve as the basis and starting point for continued state-of-the-art research. Micro-devices will be developed to rapidly detect the viability of the spores upon germination within one doubling cycle of the organisms, providing an electronic output. The devices will also have built-in electronic filters to concentrate the spores and cells inside the biochips at the detection sites. In parallel, biological analysis will be performed to identify the spore coat proteins, especially those novel to *B. anthracis* spores. These proteins are surface localized and thus useful for spore detection. The eventual system is envisioned to capture only the spores or pathogens of interest inside the bio-chips using these surface protein receptors, concentrate these microorganisms inside this chip, and electronically detect their viability and germination, while reducing the total time to result to less than possible by any other technology.

**Grant:** 1R21EB000984-01  
**Program Director:** KORTE, BRENDA  
**Principal Investigator:** AUSTIN, DAVID J PHD  
**Title:** Rapid Identification of Drug Targets in *Yersinia pestis*  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2002/09/24-2004/08/31

**DESCRIPTION** (provided by applicant): This project proposes to investigate the potential use of Display Cloning to rapidly identify and isolate the genes of protein-based therapeutic targets from biological warfare agents. This application will utilize the genome of *Yersinia pestis*, a Category A biological agent of potential use in biological warfare, to develop a panel of small-molecule probes that can be used in combination with gDNA phage display to identify genes of basic biological function and potential therapeutic importance. Once established, these probe panels can be used to provide a rapid-response approach for the target-directed drug development of therapeutic agents for novel and/or engineered biological warfare agents of known or unknown origin. Display Cloning is a procedure that combines small-molecule affinity chromatography with cDNA phage display. By utilizing a gDNA library, whole genomes of bacterial organisms can be displayed simultaneously, resulting in the ability to isolate genes from biological organisms, based on the functional ability of their resulting protein product to bind a small molecule. If the small molecule probe is a known transition-state analogue, putative protein function can be assigned. If the small molecule is a known enzyme inhibitor or drug, a therapeutic value can be ascribed to the target. An important benefit of this procedure is that not only the identity of the gene, but the gene itself is isolated. Additionally, on-phage binding analysis has been demonstrated, providing an immediate in vitro binding assay that can be used for target-based drug development. This method has been successfully utilized in mammalian systems to identify and isolate the genes of drug targets, signal transduction proteins, as well as RNA and carbohydrate binding proteins. Creating a gDNA library and performing a phage selection can be completed in less than one week. This is advantageous compared to the months or years that it may take to sequence the entire genome of an unknown or engineered organism. While microarray technology is a powerful method of looking at whole genomes, it cannot provide the functional information that can be obtained from a transition-state analogue affinity experiment. In addition to providing a starting point for drug development, analysis of biowarfare organisms with our probe panels will provide a functional taxonomic analysis of these organisms, well before their genomes can be sequenced or gene-chips constructed.

**Grant:** 1R21EB000985-01  
**Program Director:** KORTE, BRENDA  
**Principal Investigator:** HEFFRON, FRED L PHD  
**Title:** Rapid Identification of Secreted of F tularensis Antigen  
**Institution:** OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR  
**Project Period:** 2002/09/10-2004/08/31

DESCRIPTION (provided by applicant): Intracellular bacterial pathogens secrete proteins after infection that function to alter the normal structural and metabolic machinery of the host cell thus facilitating their survival and avoidance of host immune surveillance. Recent discoveries of the molecular mechanisms that intracellular bacterial pathogens use for evasion or subversion of the immune system of the host will greatly facilitate the development of antibacterial vaccines and diagnostic tools. Much like many other intracellular facultative pathogens such as Salmonella typhimurium, Mycobacterium tuberculosis and Legionella pneumophila, F. tularensis shares a predilection for macrophages as its preferred host cell. However, in contrast to other intracellular bacterial pathogens, little is known about virulence factors used by F. tularensis internally within its host cell. We have designed and tested a system that enables us to identify Class I Accessible Proteins (CAPs) in Salmonella typhimurium and propose to utilize a similar approach for the study of F. tularensis. Due to their susceptibility to the host's processing and presentation pathways, CAPs represent a potentially important resource for the design and construction of effective vaccines against F. tularensis.

**Grant:** 1R21EB000987-01  
**Program Director:** HARMON, JOAN T.  
**Principal Investigator:** BRYERS, JAMES D  
**Title:** Biomaterials that Promote Healing & Prevent Infection  
**Institution:** UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT  
MED/DNT  
**Project Period:** 2002/09/10-2005/08/31

DESCRIPTION (provided by applicant): Several prokaryotic and eukaryotic intra- and inter-cellular processes are initiated and controlled by a communication pathway from stimulus, to cell surface receptor, to cell nucleus, to mRNA, to cytokine signaling agents and higher tissue response. We recognize that both prokaryotic and eucaryotic biological processes can be influenced through cell membrane receptor mechanisms. With NIH support, we will develop model cardiovascular biomaterials that (a) use surface-tethered ligands to promote macrophage adhesion and instigate a healing cascade [1-2], while (b) releasing bioactive molecules specifically selected to negate bacterial receptor-mediated adhesion. We have developed poly(ether urethane) materials, modified with poly(ethylene glycol) tethers that (1) eliminate random protein adsorption and (2) allow for surface modifications by cell adhesion promoting peptides and monoclonal antibody fragments. While all treatments promoted macrophage adhesion, some also promoted macrophage activation. Results also indicate that both peptide- and Mab-decorated PEU surfaces significantly enhanced bacterial adhesion and biofilm formation versus base material. Consequently, for biomaterials to attract macrophage without promoting bacterial infection, a means to negate bacterial adhesion is needed. Thus, we will over a three (3) year period, develop model biomaterials that biologically prevent bacterial colonization. Objectives are: 1. We will isolate and characterize the cognate receptor(s) that the bacterium, *Staphylococcus epidermidis* (SE), employs to bind to fibronectin (FN)-coated surfaces - i.e., FN binding receptors (FN-BR) - and we will generate monoclonal antibodies (Mabs) to the entire receptor and its FN-binding domains. 2. A single chain variable fragment (scFV) antibody will be engineered from the variable heavy and light binding domains of the monoclonal antibody (MabFNBR) produced above. We will verify that the scFV antibody (FVFNBR) has the ability to bind to the SE FNBR receptor and block SE bacterial adhesion. 3. PEU materials will be fabricated containing one of the bacterial adhesion receptor blocking molecules (the MabFNBR or scFV antibody FVFNBR). Rates of bacterial anti-adhesion molecule release as a function of the amount of therapeutic agent loaded and biomaterial preparation will be determined. SE bacterial attachment studies will be carried out as a function of the specific biomaterial preparation in question and fluid phase cell concentration; under controlled hydrodynamic conditions. We will quantify macrophage adhesion, cytokine production, and macrophage activation; both with and without the presence of bacteria.

**Grant:** 2R01EY011288-04A1  
**Program Director:** SHEN, GRACE L  
**Principal Investigator:** MEDOF, M E MD  
**Title:** Protection of Ocular Tissues During Inflammation  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 1996/09/30-2007/03/31

DESCRIPTION (provided by applicant): While defending against pathogens that enter its open environment, the eye must protect itself from self cell damage that would impair visual acuity. In earlier work, we found that the decay accelerating factor (DAF or CD55), membrane cofactor protein (MCP or CD46), and the membrane inhibitor of reactive lysis (MIRL or CD59), three surface proteins that play critical roles in protecting blood cells from autologous complement-mediated injury are both ubiquitously expressed intraocularly and present in unusually high levels on the ocular surface. In the previous grant cycle, we showed that functionally active soluble forms of the molecules are present in tears and aqueous humor, and obtained evidence that changes in the cell-associated regulators are clinically relevant in ocular infections. Although these proteins have received limited study to date, an understanding of their physiological importance is beginning to emerge, the most important finding being that in a rat model the localized blocking of certain regulator activities in the conjunctiva or the anterior chamber can result in massive inflammation as well as frank tissue necrosis. One main obstacle to fully studying the regulators has been the lack of appropriate experimental animal models allowing for direct analysis of regulator functions throughout the eye in pathologic processes. In recent work, we have 1) developed a knockout mouse for one of the regulators, DAF, and 2) obtained preliminary data in a bacterial keratitis model in this knockout that neutrophil infiltration and destruction of ocular tissues are dramatically increased. In another line of studies of complement regulation, we have 1) performed exploratory studies in the eye of C5a receptors (C5aR), a newly-described membrane element that activates cellular responses and is operative in multiple brain cell types, 2) unexpectedly found that C5aR are present on retinal pigment epithelial (RPE) cells as well as corneal cells, and 3) shown that C5a ligation of C5aR on RPE cells induces the release of a number of cytokines. In this application we propose to 1) exploit our DAF knockout mouse in conjunction with CD59 knockouts developed by our collaborators to precisely define the physiological importance of the regulators in ocular infections, and 2) fully characterize the functions of C5aR on RPE and corneal cells and examine their role in immunological processes. For this work, in addition to available human and mouse antibodies, mouse knockouts will be utilized. The studies should provide new insights into how both complement regulators and C5aR serve to protect the eye, new knowledge about RPE cells and corneal cells, and new information regarding host defense mechanisms in ocular infections.



**Grant:** 1R03EY013782-01A1  
**Program Director:** SHEN, GRACE L  
**Principal Investigator:** WILHELMUS, KIRK R MD  
**Title:** Ophthalmic Antibiotic Resistance Study  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2002/08/01-2004/07/31

**DESCRIPTION:** (Applicant's Abstract) Microbial keratitis is a common, economically important ocular disease that, despite the availability of antimicrobial therapy, can reduce vision and the quality of life. A major unresolved issue is the role of the microbiology laboratory in guiding decision-making. The goal of this pilot research is to use clinical and microbiologic data from patients who have had microbial corneal infection to determine how antimicrobial resistance affects outcome. Using a retrospective cohort study design, this epidemiological investigation will examine the effect of the appropriateness of initial antibiotic therapy and the in vitro susceptibility profile of corneal isolates on vision, length of antibiotic treatment, and ocular complications requiring surgery. Multivariable logistic-regression and proportional-hazards analyses will control for potential confounders (including demographic variables, disease duration, severity parameters, and adjunctive therapy) and will consider possible effect modifiers (such as microbial taxonomy and minimal inhibitory concentration) to determine adjusted relative effect measures. The knowledge derived from this research has a potentially high impact on vision research and on clinical practice and has the potential to direct broader investigations into the problem of antimicrobial resistance and the optimal use of antibiotics in ophthalmology.

**Grant:** 2P01GM037696-16  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** ULEVITCH, RICHARD J PHD  
BIOCHEMISTRY:PROTEIN/A  
INO ACID  
**Title:** BIOCHEMICAL MECHANISMS OF CELLULAR INJURY IN TRAUMA  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 1986/12/01-2007/07/31

DESCRIPTION (provided by applicant): Regardless of the origin of the infection, septic shock continues to be a serious complication for burn and trauma patients. Most investigators acknowledge the importance of bacterial products like endotoxin (LPS) in responses of the innate immune system that contribute to septic shock. Members of this Program Grant have provided seminal findings that led to the appreciation of the importance of two proteins, LPS binding protein (LBP) and CD14, in the initiation of these host responses to LPS. LBP functions as a transfer protein delivering LPS to CD14, a 55-kDa glycoprotein found in two forms; a membrane protein (mCD14) of myeloid lineage cells and a soluble plasma protein (sCD14). mCD14 is important in activation of myeloid lineage cells while sCD14 participates in activation of non-myeloid cell types. Cellular responses to LPS are initiated at the cell membrane and culminate with intracellular kinase cascades which regulate changes in gene expression and promote other cellular responses. A full understanding of intracellular signalling pathways used by LPS still represents a substantial gap in our knowledge. Here we focus on bridging what we believe to be the most significant gaps in our knowledge about LPS/CD14-dependent pathways of cell activation. These studies will combine approaches involving structural, molecular and cell biology to address key questions regarding the regulation of LBP biosynthesis, the structure and function of CD14, the earliest steps of LPS induced signal transduction, the basic mechanisms involved in LPS-induced activation of oxidant production and the role of the LBP/CD14 dependent pathway in animal models of septic shock and in clinical disease in man. Collectively these projects promise to provide advances in our understanding of this pathway that are likely to yield new approaches to treating septic shock in man.

**Grant:** 2P01GM056531-06

**Program Director:** CASSATT, JAMES

**Principal Investigator:** ORTIZ DE MONTELLANO, PAUL R PHD  
CHEMISTRY:CHEMISTRY-  
UNSPEC

**Title:** Structure Biology and Targeted Drug Design for AIDS

**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO  
SAN FRANCISCO, CA

**Project Period:** 1997/09/01-2007/08/31

DESCRIPTION (provided by applicant): The continued structure-based development of inhibitors of key proteins of the human immunodeficiency virus (HIV) and of two organisms responsible for HIV-related opportunistic infections is proposed. Despite major advances in the treatment of acquired immunodeficiency syndrome (AIDS), it remains a major threat to the public health. Furthermore, the widespread appearance of resistance to antibacterial and antiviral drugs and the escalating costs of drug discovery and health care make the development of more rapid and efficient drug discovery methods imperative. Three HIV proteins, integrase, Rev, and Tat, are to be targeted for inhibitor development, and the mechanism of resistance to inhibitors of the HIV-1 protease is to be investigated. In addition, two AIDS-related opportunistic infections, Kaposi's sarcoma and drug-resistant tuberculosis, will be targeted. Discovery efforts will focus on (a) the protease of HHVS, the virus responsible for Kaposi's sarcoma, (b) the Mycobacterium tuberculosis alkylhydroperoxidases AhpC and AhpD that compensate for loss of the KatG peroxidase in isoniazid resistance, and (c) EtaA, the M. tuberculosis flavoprotein that activates ethionamide. The development of inhibitors of KasA-AcpM, one of the ultimate targets of activated isoniazid and ethionamide, is proposed. The proteins required for these studies are to be produced by recombinant methods and purified, crystallized, and subjected to X-ray diffraction analysis. Mechanistic studies of the less well characterized enzyme targets will be carried out to obtain the information required for the design of reversible and irreversible inhibitors. Structural and mechanistic information will be used in conjunction with computational methods to identify potential inhibitors. The inhibitor candidates will be synthesized, assayed with isolated enzymes, and in some cases co-crystallized with the enzymes for structural analysis. Inhibitor optimization will be assisted by computational approaches, and the improvement of such approaches for the discovery and optimization of drug candidates is a further goal of this research program. Promising drug candidates will be evaluated in cell culture and in vivo. This broad, structure-based attack on HIV and two important opportunistic infections should produce fundamental knowledge relevant to the functions of the proteins investigated, to our understanding of drug resistance, to the design of drugs for infectious agents, and to useful drug leads for AIDS.

**Grant:** 2R01GM013598-37  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** TROST, BARRY M PHD CHEMISTRY:ORGANIC  
**Title:** Synthesis of Macrolides. Steroids, Cyclopentanoids, etc  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 1987/06/01-2006/05/31

**DESCRIPTION:** (provided by applicant) Exploring biological phenomena at a molecular level provides the basis of understanding from which new therapeutic agents derive. The ability to construct a defined molecular architecture requires highly selective reactions and reagents to permit the development of effective synthetic strategies. Cyclic compounds have biological activities across a broad spectrum. Furthermore, constraining conformations of mobile molecules by forming rings also frequently enhances biological potency. Thus, a concerted effort to apply new chemical principles being developed in these laboratories to the formation of rings becomes an important objective. In the first domain, a new strategy to effect cyclizations, in general, and macrocyclizations, in particular, asymmetrically may provide a unique opportunity to approach a variety of significant targets. The concept involves a new dinuclear catalyst design for an asymmetric aldol addition that involves no preactivation of either partner (i.e., no stoichiometric formation of an enol or enolate) and that could also provide an unusual ability to perform macrocyclizations at high concentration. This development leads to the use of hydroxyacetone as a lynchpin to form macrocycles asymmetrically and a synthesis of the antitumor amphidinolides. In the second domain, the development of a new annulation to form heterocycles leads to a novel convergent and practical approach to the potent antitumor agents, the bryostatins, and potential analogues. The asymmetric aldol reaction also will play an important role. This domain embodies an atom economic cross-coupling of two different alkynes. This new concept for C-C bond formation performed intramolecularly sets the stage for macrocyclizations that can lead to the cochleamycins, new structural class of antitumor antibiotics. The third domain embodies a new class of cycloaddition reactions to create odd membered rings. Exploring a new class of acceptors in conjunction with a novel class of reactive intermediates creates a conceptual framework to the anthelmintic and antinematodal mold metabolites paraherquamide and marcfortine. An unusual (6+3) cycloaddition may create strategies for the structurally unusual farnesyl transferase inhibitor CP-263,114 and simple analogues. A new bifunctional conjunctive reagent combined with a ring expansion provides a novel approach to the antitumor agent penostatin 1. A (4+3) cycloaddition combines with two other concepts being developed in these laboratories, asymmetric allylic alkylation and metal catalyzed enyne cycloisomerizations, to create a powerful strategy to the neurotrophic agents, the erinacines. The fourth domain transitions to ruthenium catalysis for (5+2) cycloadditions. This new concept sets the stage for solutions to a long standing problem, the ion channel blockers, the grayanotoxins, as well as the more recently discovered rameswaralide, a potent antiinflammatory.

**Grant:** 2R01GM015792-36  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** VON HIPPEL, PETER H PHD  
BIOCHEMISTRY:PHYSICAL  
**Title:** Structure and Relations of Proteins and Nucleic Acids  
**Institution:** UNIVERSITY OF OREGON EUGENE, OR  
**Project Period:** 1978/01/01-2005/12/31

In this grant application we describe plans to continue our ongoing studies of the molecular mechanisms and regulation of the transcription complex of *E. coli*. We will focus, in particular, on how the complex is regulated by transcription factors, building on the recent major progress that has greatly increased our understanding both of many aspects of the structure of the complex, as well as of the thermodynamics and kinetics of the control of the reactions that lead to transcript initiation, elongation, editing, and termination. This later progress, in particular, makes it possible for us to begin to develop quantitative insight into the changes that the binding of transcription factors must bring about to (e.g.) redirect the transcription process from elongation to transcript editing or termination. During the next reporting period our Specific Aims will be: (i) to continue our studies of the mechanisms whereby the N protein-dependent antitermination system of phage lambda controls the transcription termination efficiency of *E. coli* RNA polymerase at intrinsic and rho-dependent terminators; (ii) to carry out fundamental studies of RNA flexibility and looping as a component of transcription regulation; (iii) to elucidate the role of specific RNA sequences and *E. coli* host factors in regulating the "range" and specificity of "full" transcription factor complexes involved in N-dependent antitermination; (iv) to determine the detailed mechanism of action of *E. coli* transcription termination factor rho at rho-dependent terminators, and to study the roles of "coupling factors" in regulating the efficiency of the rho-dependent termination process; (v) to examine the interconversion of the various forms of the *E. coli* transcription elongation complex that lead, respectively, to elongation, editing, and termination, as well as to use rho as a probe of these "functional states"; and (vi) to perform theoretical and modeling studies of the assembly and stability of functional complexes of transcription factors to better understand how these complexes assemble, and how the components interact to build a stable "macromolecular machine". In terms of their significance for biomedical research, these studies will serve as molecular models for the function and control of the analogous DNA-dependent RNA transcription systems of higher organisms, and may help reveal how these controls can go awry in cancer and other diseases of inappropriate gene expression.

**Grant:** 2R01GM019416-31

**Program Director:** ANDERSON, RICHARD A.

**Principal Investigator:** HOCH, JAMES A PHD  
MICROBIOLOGY:MICROBIO  
OGY-UNSPEC

**Title:** Genetic Control of Development

**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA

**Project Period:** 1977/05/01-2006/06/30

DESCRIPTION (provided by applicant): Bacteria are highly adaptable organisms capable of growth on countless carbon and nitrogen sources and of occupying an inexhaustible variety of ecological niches including, unfortunately, regions of the human body that are better left bacteria-free. The key to adaptability in bacteria is their capacity to express only those genes for enzymes and pathways that they need for maximal growth in the environment in which they find themselves. One of the major mechanisms of signal recognition leading to specific gene expression is the two-component system and its more complex variant, the phosphorelay. This proposal has the goal of understanding the mechanisms by which both an essential two-component system and the sporulation phosphorelay function, from signal ligand identity to molecular recognition between components and gene activation. Genetic methods will be used to identify signals and proteins activating sporulation sensor kinases. Proteins associating with sensor kinases will be identified. The YycG sensor kinase and YycF response regulator are essential for growth. The genes regulated by this two-component system will be identified by microarray analysis and bioinformatic techniques. Suppression, transposon and multi-copy gene expression techniques will be used to identify the role of the YycF response regulator in gene expression. The role of amino acid side chains in the surface of interaction of response regulators and phosphotransfer domains in recognition specificity will be studied by modifying the Spo0A transcription factor to a sensor kinase substrate. The structure and function of the KinA domains will be studied by domain liberation using mutants bearing specific proteolytic sites in interdomain region. Experiments are proposed to test the hypothesis that the dynamics of the loops making up the active site of Spo0F is an important determinant of sensor kinase specificity and that the dynamics may be influenced by regulating ligands that bind to the response regulator. It is believed that this combination of structural and functional studies will lead to effective anti-bacterial agents either directly or in combination as inhibitors of resistance mechanisms.

**Grant:** 2R01GM020194-30  
**Program Director:** FLICKER, PAULA F.  
**Principal Investigator:** CHASTEEN, NORMAN D PHD CHEMISTRY:PHYSICA  
**Title:** Iron Deposition and Mobilization in Ferritin  
**Institution:** UNIVERSITY OF NEW HAMPSHIRE DURHAM, NH  
**Project Period:** 1975/06/01-2006/06/30

DESCRIPTION (provided by applicant): From microorganisms to mankind, ferritin plays a central role in the biological management of iron. The ferritins function as iron storage and detoxification proteins by depositing iron as a hydrous ferric oxide mineral within their shell-like structures. This iron can be subsequently mobilized for the synthesis of heme. While ferritins from various organisms share many common structural features, being either 12 or 24 subunit proteins, they differ markedly in their chemistries of iron deposition. All known ferritins contain ferroxidase sites that catalyze iron(II) oxidation by either molecular oxygen or hydrogen peroxide; however they do so in significantly different ways. This proposal focuses on the mechanisms of iron deposition in a variety of recombinant 24mer ferritins that include human H- and L-chain ferritins, a newly discovered human mitochondrial ferritin, the heme-containing E. coli bacterioferritin (EcBFR) and the E. coli northeme bacterial ferritin (EcFtnA). Studies will also be conducted with the 12mer proteins: Listeria innocua ferritin and the DNA binding protein, Dps from E. coli. Important questions relating to dioxygen binding, ferritin-ferritin association during iron oxidation, transient radical and iron intermediates and the stoichiometric equations for iron oxidation and hydrolysis using dioxygen and hydrogen peroxide as oxidants will be addressed for the different proteins. Features of the mechanisms of iron deposition of the various ferritins will be elucidated through a combination of site-directed mutagenesis in conjunction with x-ray structure data, isothermal titration calorimetry, UV-visible stopped-flow kinetics, rapid-freeze quench Mossbauer and EPR spectroscopies, spin trapping, light scattering, oximetry and pH stat. The extensive studies proposed should lead to a detailed understanding of how various ferritins function as reversible iron storage proteins, assisting the cell cope with oxidative stress, and further our knowledge of the chemistry and biochemistry of iron biomineralization processes in general.

**Grant:** 2R01GM028470-22

**Program Director:** ANDERSON, RICHARD A.

**Principal Investigator:** GRINDLEY, NIGEL D PHD  
GENETICS:BIOCHEMICAL/M  
LECULAR

**Title:** Mechanism of Insertion Sequence Translocation

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

**Project Period:** 1980/05/01-2006/04/30

DESCRIPTION (Provided by applicant): The overall goal of this project is to understand, in as much detail as possible, the mechanisms of two different kinds of specialized recombination associated with transposable DNA elements: site-specific recombination and transpositional recombination. The model system for site-specific recombination - a breakage-exchange-reunion reaction between two specific sites - is the resolution of cointegrates (the product of transpositional recombination by the gamma delta transposon) by the gamma delta resolvase, prototype of the serine recombinases. Transpositional recombination will be studied using a transposon of the D,D(35)E superfamily, Tn552. Site-specific recombination mediated by the gamma delta resolvase. A primary goal is to elucidate the architecture of the synaptic complex (containing two 120 bp ressites and 6 dimers of resolvase) within which recombination occurs. We have formulated a new structural model for this complex based on crystallography of the resolvase dimer and a detailed knowledge of interdimer interactions. A variety of approaches, particularly fluorescence resonance energy transfer (FRET), will be used both to test predictions of this (and an opposing) model, and also to probe for large rearrangements of the resolvase subunits or domains that accompany the activation of catalytic functions and the process of strand exchange. An investigation of another related serine recombinase, the transposase of the *Helicobacter pylori* element, IS607, will also be initiated. Transpositional recombination by Tn552. We have developed an efficient in vitro strand transfer reaction for Tn552, using the TnpA transposase and a transposon substrate with pre-cleaved ends. We intend to focus on the role of the accessory transposition protein, TnpB in activation of TnpA (allowing it to cleave uncleaved transposon ends) and in transposition immunity.



<b>Grant:</b>	2R01GM028961-21	
<b>Program Director:</b>	SCHWAB, JOHN M.	
<b>Principal Investigator:</b>	KECK, GARY E	PHD CHEMISTRY:ORGANIC
<b>Title:</b>	Chiral Approaches to Natural Product Synthesis	
<b>Institution:</b>	UNIVERSITY OF UTAH	SALT LAKE CITY, UT
<b>Project Period:</b>	1981/06/01-2006/03/31	

DESCRIPTION: (provided by applicant) The long term goals of this program include the laboratory synthesis of complex natural products possessing desirable biological activity as well as the development of new synthetic methodology that will simplify this task. In addition, we plan to attempt to identify, and to synthesize and test, analogs of these naturally occurring lead compounds which can be accessed synthetically in a more practical manner than the natural compounds themselves. Ongoing investigations on the total synthesis of the complex cytotoxic macrodiolide swinholide will be continued and hopefully brought to completion, as will studies on the promising compound epothilone, which has activity similar to that of taxol. Our studies on the potent and extremely promising anticancer agent bryostatin 1 will continue, and we hope to expand this program aggressively into analog synthesis. Syntheses of leucascandrolide and dolabelide B will be initiated, and analogs of leucascandrolide will be prepared for assay as antifungal and cytotoxic agents. Throughout all of this work, we hope to implement new organic reactions and synthetic strategies which will facilitate the construction of the targeted compounds, as well as prove useful in a broader context.

**Grant:** 2R01GM029028-20A1  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** SMITH, AMOS B  
**Title:** Synthesis of Bioactive Natural Products  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 1991/09/01-2006/02/28

**DESCRIPTION:** (provided by applicant) The principal goals of this research program for the next four years are: (A) to complete the total synthesis of the anticancer agents (+)-tedanolide and (+)-13-deoxytedanolide, exploiting a unified synthetic strategy; (B) to devise an enantioselective total synthesis of the potent insecticidal agent (+)-nodulisporic acid; and (C) to achieve a total synthesis of the novel, architecturally challenging macrolide antibiotic sorangicin A. In addition, new innovative directions for our dithiane chemistry will include: (D) reaction of dithianes with nitrogen-containing electrophiles (e.g., aziridine and allylic aziridines) for the development of new multicomponent assembly tactics; (E) reaction of dithianes with allylic epoxides, exploiting the SN2 and SN2' addition manifolds; and (F) merged SN2 and SN2' linchpin dithiane couplings. Finally, we will (G) showcase the multicomponent linchpin coupling of 2-lithio-2-trialkylsilyl-1,3-dithianes with aziridines and vinyl epoxides, respectively with expedient total syntheses of the Mantella alkaloid 223 AB and the aglycon of (+)-rimocidin. Beyond these specific synthetic objectives, a general, long-range goal of this program is the identification of the molecular architecture responsible for biological activity. Thus, as we develop an approach to each target structure, we will also prepare model compounds designed to permit the elucidation of structure-activity relationships.

**Grant:** 2R01GM031030-21

**Program Director:** ANDERSON, JAMES J.

**Principal Investigator:** WALKER, GRAHAM C PHD  
BIOCHEMISTRY:NUCLEIC  
ACID

**Title:** Molecular Genetics of Rhizobium Nodulation Plasmids

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

**Project Period:** 1982/07/01-2006/07/31

DESCRIPTION (provided by applicant): A long-term goal of this research is to understand the molecular mechanisms by which *Sinorhizobium meliloti* invades the nodules that it elicits on its plant hosts and establishes a productive symbiosis. Another long-term goal is to use knowledge gained from this research to increase our understanding of other bacterial-host interactions, particularly those involving chronic infections of mammals by bacterial pathogens. We have already shown that the synthesis of any of three different *S. meliloti* extracellular polysaccharides (succinoglycan, EPS II, and K antigen) in a symbiotically active low molecular weight form is required for invasion through infection threads. We will further characterize the quorum sensing regulation of these exopolysaccharides, examine the regulatory role of ExoS-ChvI, and further analyze their biosynthetic mechanisms. We will carry out studies of how the symbiotically active forms of the exopolysaccharide interact with the plant and will test how alteration of the chemical structure, non-carbohydrate modification, and molecular weight distribution of exopolysaccharide influence the architecture of biofilms. We have shown that *S. meliloti*, a plant symbiont, and *Brucella abortus*, an animal pathogen, both require the function of the *bacA* gene for the chronic intracellular infections they cause in their respective hosts. The proposed research will determine the molecular basis of *bacA* function, test the importance of *bacA* in other chronic host-pathogen interactions, and further explore possible commonalities between *S. meliloti* symbiosis and *B. abortus* pathogenesis. By taking advantage of the recent sequencing of the *S. meliloti* genome, we have identified 30 genes previously unrecognized as being important for symbiosis and will further study the roles of the most interesting of these. Our work demonstrating the importance of *BacA* protein in *Brucella* chronic infections has identified it as a possible target for new classes of drugs active against chronic infections. There is no human vaccine for *Brucella*, a potential bioterrorism threat, and *B. abortus bacA* mutants are potential vaccines. Our work will continue to offer insights into how specific low molecular weight oligosaccharides can serve as signals to eukaryotic hosts.

**Grant:** 2R01GM031657-19  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** COZZARELLI, NICHOLAS R PHD BIOCHEMISRY  
**Title:** Enzymology and Genetic Studies of DNA Polymerases  
**Institution:** UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA  
**Project Period:** 1982/07/01-2006/06/30

DESCRIPTION (provided by applicant): This project focuses on three giant motor proteins, topoisomerases, helicases, and FtsK that move DNA through large distances utilizing the energy of NTP hydrolysis and mechanical strain on DNA. We hope to understand how these proteins perform these vital roles in DNA replication and chromosomal segregation. We will use single DNA molecule enzymology complemented with bulk measures. The action of a single enzyme acting on DNA is measured by the resultant changes in DNA force, torque, and extension. The single DNA molecules can be supercoiled or braided at will to generate substrates for the enzymes. We will measure the rates of enzyme action, processivity, stall force, and chirality in interaction with superhelical DNA. These results will then be compared with bulk measures and measures in vivo. The clear medical relevance stems primarily from two sources. First, topoisomerases are the favored targets of antibiotics such as ciprofloxacin, and anticancer agents, such as etoposide and adriamycin. The understanding of their unusual dominant poisoning of their targets has greatly aided the development of more potent drugs. Second, interference in proper segregation of chromosomes by mutations that affect motor proteins accompanies and exacerbates human diseases, including cancer and premature aging.

<b>Grant:</b>	2R01GM032618-30A1	
<b>Program Director:</b>	ANDERSON, JAMES J.	
<b>Principal Investigator:</b>	NESTER, EUGENE W	PHD MICROBIOLOGY:MICROBIO LOGY-UNSPEC
<b>Title:</b>	Molecular Basis of Crown Gall Tumorigenesis	
<b>Institution:</b>	UNIVERSITY OF WASHINGTON	SEATTLE, WA
<b>Project Period:</b>	1983/05/01-2006/02/28	

DESCRIPTION (provided by applicant): The long-term objective of this proposal is to understand in molecular terms the mechanism by which *Agrobacterium tumefaciens* interacts with its host resulting in Crown Gall tumor formation in a wide variety of plants. This proposal follows the same general approach that we have used in previous years to gain insight into these mechanisms, namely, to analyze mutants altered in tumor formation. However, the experiments described are much more focused because we now know the sequence of the *Agrobacterium* genome and we have access to microarray chip technology. With this new information and technology we can carry out directed mutant studies and types of analyses that were not possible previously. The information revealed by the sequence of the genome has raised questions but also the means to answer these questions which we could only speculate on previously. Thus, we now know that far more genes are activated in *Agrobacterium* by interaction with host plants than was recognized previously. Using microarray chip technology we will identify and characterize global regulatory circuits that are directly activated or suppressed by plant signal molecules. Using microarray technology, we will expand on previous observations by determining the alterations in the global expression of genes in *Agrobacterium* grown with plant cells at a temperature optimum for the growth of *Agrobacterium* but inhibitory for the transfer of T-DNA (28x). We will also continue our studies on the mechanism by which T-DNA is transferred from *Agrobacterium* into host cells, focusing on the role of VirJ in this process. Finally, the genome sequence of *Agrobacterium* has revealed many genes that serve pathogenicity functions in related bacteria. We will mutate these genes to determine if they are also required for pathogenicity of *Agrobacterium*. The studies proposed in this application should open up new vistas and provide an increased understanding of the many levels of interaction of *Agrobacterium* with its variety of eukaryotic hosts.

**Grant:** 2R01GM033143-17  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** HIGGINS, NORMAN P PHD  
**Title:** Long Range Interactions in Mu and Bacterial DNA  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL  
BIRMINGHAM  
**Project Period:** 1983/07/01-2006/06/30

DESCRIPTION (provided by applicant): DNA domain formation is critical for eukaryotic and prokaryotic cells alike. The dynamics of DNA movement inside a living cell is a central problem in biology. How DNA is twisted, turned, tangled, and untangled is a major problem that impinges on cellular enzymes that perform functions like transcription, genetic recombination, chromosome segregation, and replication. Domain regulation underpins cell development and gene regulation in organisms as diverse as man (i.e. hematopoiesis) and bacteria (i.e. in adapting to a harsh environment). A method that uses the Tn<sub>+</sub> site-specific recombination pathway has been developed to study supercoil dynamics and domain structure inside living cells. This analysis can be performed at any desired point in the bacterial genome. Using our system, several types of domain boundaries have been located. The most abundant barrier class occurs during replication and these barriers are located stochastically over the sequence with an average spacing of 30 kb. Rarer sequence specific barriers arise from transcription at very active promoter and at gene clusters that encode membrane-inserted proteins. We plan to derive the global pattern of domain structure by analyzing at least 50 test intervals that span the genomes of *E. coli* and *S. enterica* serovar Typhimurium. One aim is to see if conserved operons that encode clusters of membrane proteins are position specific barriers. There are 23 of these operons in the sequenced genomes of *E. coli* and *Salmonella* that have remained at fixed points in the drifting genomes. Second, we will identify mutants that change DNA domain structure at two critical points for controlling cell division-- the origin and terminus of DNA replication. Third, we will characterize proteins that alter DNA dynamics. The results from these studies will provide critical information about how DNA dynamics influences a wide variety of biochemical processes on DNA. They will also shed light on the mechanisms by which chromosomes achieve structural stability over deep time.

**Grant:** 2R01GM033327-16  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** EVANS, DAVID A PHD CHEMISTRY:ORGANIC  
**Title:** ASYMMETRIC SYNTHESIS OF IONOPHORE/MACROLIDE ANTIBIOTICS  
**Institution:** HARVARD UNIVERSITY CAMBRIDGE, MA  
**Project Period:** 1983/08/01-2006/03/31

The objectives of this proposed research are to make creative contributions to the total synthesis of naturally occurring substances possessing clinically significant biological activity. This grant will continue to address the development of new stereoselective reactions and the application of this methodology to the asymmetric synthesis of polyketide-derived antibiotics and anti-neoplastic agents. The synthesis targets will include aflastatin A, amphidinol 3, pectenotoxin, callipeltoside A, cochleamycin, and hexacyclinic acid. Enantioselective processes developed within this research program have been integrated into the proposed synthesis plans for the indicated target structures. The methodological studies dealing with reaction discovery will emphasize the development of reactions for controlling and ultimately predicting the stereochemical course of complex aldol processes. The ongoing goal of these studies has been to reveal some of the general rules for predicting the stereochemical outcome of complex aldol fragment coupling reactions. Along with polynucleotides, peptides and polysaccharides, polyketides represent the fourth broad family of naturally occurring materials that are assembled from common subunits. In extending the family of naturally occurring materials that are assembled through complex aldol addition reactions is a far greater challenge than the amide construction analogy. Our long-term objective has been the development all of the necessary tools for the efficient assemblage of complex polypropionate and polyacetate targets. Asymmetric catalysis has been directed to the construction of chiral building blocks. Chiral enolate methodology has been developed for stereoregulated aldol processes, and studies on double stereodifferentiating aldol reactions have revealed how to improve the design predictability of these fragment coupling reactions. Our goal has been to set in place all of the reactions necessary for the rapid assemblage of any polyketide target structure.

**Grant:** 2R01GM033476-17

**Program Director:** WOLFE, PAUL B.

**Principal Investigator:** MATSON, STEVEN W PHD  
BIOCHEMISTRY:NUCLEIC  
ACID

**Title:** Enzymatic Mechanisms of DNA Helicases

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

**Project Period:** 1984/04/01-2006/06/30

DESCRIPTION (provided by applicant): DNA helicases catalyze NTP hydrolysis-dependent unwinding of duplex DNA to provide single-stranded DNA (ssDNA) for use as a template or reaction intermediate in DNA transactions. We have focused our efforts on DNA helicases in *E. coli* and the budding yeast *Saccharomyces cerevisiae*. The long-range goal of this research program is to understand, in enzymatic and molecular terms, the mechanism of action and cellular role of several important DNA helicases in *E. coli* and yeast. Our focus during the next grant period will be on DNA helicases II and IV from *E. coli*, and Sgs1p and Hmi1p from yeast. The 1st and 2nd aims will continue our efforts to define the interaction between helicase II (UvrD) and MutL, two proteins that play a critical role in maintaining genomic stability. We will determine the mechanism by which MutL stimulates UvrD-catalyzed unwinding using biochemical approaches. In addition, we will evaluate, through genetic studies, the importance of the interaction between these proteins by identifying mutants that fail to interact. The 3rd aim proposes to identify proteins that interact with and modulate the activity of UvrD and helicase IV using a novel biochemical approach. We will biotinylate the target proteins *in vivo* and identify interacting proteins in pull-down assays from cell extracts. The effect of these proteins on the biochemical activities of each helicase will be evaluated in biochemical and genetic experiments. This will shed additional light on the roles these proteins play in the cell. The final two aims focus on two DNA helicases from budding yeast. The Sgs1p has been expressed as a full length protein in baculovirus and will be thoroughly characterized as a helicase with regard to unwinding mechanism (processive vs. distributive), substrate preferences (DNA vs. RNA vs. DNA-RNA) and substrate structure. In addition, the interaction between Sgs1p and Topoisomerase III will be investigated using biochemical methods. Preliminary studies suggest that topo III modulates the biochemical activity of Sgs1 p. These studies will provide additional information relevant to the role of Sgs1 p in maintaining genomic stability. Finally, a mitochondrial helicase, Hmi1p has been identified and partially characterized in genetic studies. A thorough biochemical description of this protein is lacking and will shed light on the role this protein plays in mtDNA metabolism. This protein will be expressed and fully characterized in biochemical assays.



<b>Grant:</b>	2R01GM034548-16	
<b>Program Director:</b>	CASSATT, JAMES	
<b>Principal Investigator:</b>	BIRGE, ROBERT R	PHD CHEMISTRY:CHEMISTRY- UNSPEC
<b>Title:</b>	Photobiology of Rhodopsin & Bacteriorhodopsin	
<b>Institution:</b>	UNIVERSITY OF CONNECTICUT STORRS	STORRS-MANSFIELD, CT
<b>Project Period:</b>	1988/08/01-2006/08/31	

DESCRIPTION (provided by applicant): The long-term objectives of this research program are to understand the molecular details of protein photochemical mediation and wavelength regulation in bacteriorhodopsin and the visual pigments of rods and cones. Our emphasis for the present grant period is to study the blue, violet and uv cones because relatively little is known about these pigments. The key spectroscopic tools that will be used include one-photon and two-photon spectroscopy, Fourier-transform infrared spectroscopy, Raman spectroscopy, CD spectroscopy and pulsed laser photocalorimetry. The principal biochemical studies to be undertaken include site-directed mutagenesis and chromophore analog substitutions as well as random mutagenesis followed by screening for wavelength and photochemical properties. The theoretical studies will rely heavily on the use of MNDOPSDCI molecular orbital theory to probe the photophysical properties of the protein-bound chromophores. An important and new goal of this grant period is to add the prediction of circular dichroism spectra to the MNDOPSDCI procedures, with the immediate goal of using experimental CD spectra to analyze the protein binding sites of rhodopsin and the cone pigments. We will also use ab-initio and semiempirical molecularorbital theory to examine the ground state properties of the chromophore binding sites while using molecular mechanics to describe the remaining portions of the protein. Our goal is to combine experiment and theory in a synergistic program that enhances both. In addition to the more global goals outlined above, we will seek to answer the following specific questions: (1) What are the principal mechanisms of wavelength regulation in the cone pigments? (2) What is responsible for the significant difference in the absorption spectra of bacteriorhodopsin versus sensory rhodopsin II? (3) What are the mechanisms through which the chloride binding sites in the cone pigments influence the spectroscopic properties of the chromophores in the long wavelength cone pigments? (4) Where are the calcium binding sites in bacteriorhodopsin, and how do these sites influence the photophysical properties of the bound chromophore? (5) What specific proteinchromophore interactions are responsible for selecting 6-s-cis versus 6-s-trans ring conformations of the bound chromophore, and why does rhodopsin select the 6-s-cis while bacteriorhodopsin and sensory rhodopsin select the 6-s-trans conformation?

**Grant:** 2R01GM034766-16  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** TAI, PHANG-CHENG PHD  
MICROBIOLOGY:MICROBIOLOGY  
PHYSIOLOGY  
**Title:** Protein translocation across Escherichia coli membranes  
**Institution:** GEORGIA STATE UNIVERSITY ATLANTA, GA  
**Project Period:** 1985/07/01-2006/01/31

**DESCRIPTION** (provided by applicant): The localization of proteins to different cellular and extracellular compartments to carry out various functions is of fundamental importance to all living cells. This project will center on the general secretion pathway (the Sec pathway) Escherichia coli which involves SecA, SecB, SecY, SecE, SecG, SecD, SecF and YajC. We will continue to combine molecular manipulation and biochemical studies in our well established in vitro systems with E. coli inverted membrane vesicles. In addition, we will extend the complementary electrophysiological and physical approaches that are newly developed for further studies. Building on the recent unexpected findings, though if somewhat against the current dogma, that some SecA integrates into membranes and does not cycle on and off membranes during translocation, we will test the hypothesis that in addition to catalyzing ATP hydrolysis, certain domains of SecA play an important structural role in the translocation machinery, forming part of the protein-conducting channels. This hypothesis is gaining further support with the recent findings that ionic current activity of the protein-conducting channels can be observed in the absence of SecYEG complex, and that SecA alone forms ring-like and dumbbell structures upon interaction with anionic phospholipids. These new exciting findings in the context of how SecA functions in the membranes, as well as the roles of SecYEG in protein translocation will be further explored in this project. The specific aims are: 1.) To further characterize the functions of membrane SecA in the Sec secretion pathway: (a) to determine the function of lipid-specific domains of SecA in the membranes; (b) to determine the role of SecA in electro-current activity of the protein conducting channel; (c) to determine the structures of SecA upon interaction with phospholipids; and 2). To determine the roles of SecYEG and other proteins in protein translocation: (a) to determine the functions of SecYEG; (b) to determine the roles of other proteins; and (c) to test a simplified working model. Characterizing the roles of SecYEG and SecA in the membranes as proposed here is of fundamental significance to understanding the mechanisms of bacterial protein secretion, which has important medical and industrial applications.

**Grant:** 2R01GM036925-14A1  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** PEARSON, ANTHONY J PHD CHEMISTRY:ORGANIC  
**Title:** Arene Activation by Transition Metals  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 1986/07/01-2005/03/31

DESCRIPTION: (provided by applicant) The objectives of the proposed research are to use in organic synthesis the ability of transition metals to activate aromatic substrates toward nucleophilic addition. Two main areas of endeavor will be studied: (1) Chromium tricarbonyl complexes of alkoxybenzene derivatives, in which the alkoxy group is chiral, will be used as substrates for asymmetric carbon nucleophile addition reactions. The outcome of this protocol is the formation of chiral substituted cyclohexenones in high enantiomeric excess. (2) Cyclopentadienylruthenium complexes of chloroarenes will be used to effect nucleophilic aromatic substitution as a key step in the total synthesis of the aglycone of ristocetin A, which is a complex peptido aryl ether related to the important glycopeptide antibiotics vancomycin and teicoplanin.

**Grant:** 2R01GM038922-15  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** BECKWITH, JONATHAN R PHD  
**Title:** MEMBRANE PROTEINS INVOLVED IN CELL DIVISION IN E.COLI  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 1987/09/01-2006/02/28

DESCRIPTION (Provided by applicant): This proposal is designed to yield new information on the process of cell division in the bacterium *Escherichia coli*. Understanding the details of this process is important in designing new classes of antibiotics that can be used in the treatment of bacterial infections. We are currently focusing on two cytoplasmic membrane proteins required for cell division, FtsL and FtsQ. We wish to determine the features of these proteins that cause them to localize to the cell division site. We will obtain a collection of dominant negative mutations in the *ftsQ* and *ftsL* genes. Localization of these mutant proteins to mid-cell will be determined with green fluorescent protein (GFP) fusions, using fluorescence microscopy. The dominant-negative mutants will be used to select for mutations that fail to localize to the cell division site. These mutations will help define the region(s) of these proteins that direct them to that site. Mutations in other genes or multi-copy plasmids that suppress the localization defects in FtsQ and FtsL may reveal other proteins these proteins interact with. Multi-copy suppressors of the dominant mutants themselves could reveal the identity of interacting proteins. Protein-protein interactions will be further probed by examining the functioning in *E. coli* of pairs of proteins from closely-related species of bacteria. A newly discovered gene will provide a test case of this approach. We will use genetic analysis with bioinformatic approaches to help uncover the function of the FtsQ and FtsL proteins. The dominant-negative and other mutations of *ftsQ* should yield information on the functional regions of the protein. Defining residues important for the activity of this protein would point to particular regions of the protein to study further. We would then continue our computer searches of databases for proteins that share small regions of homology with such regions of FtsQ. In addition, suppressors of an *ftsQ* null mutant, if obtained, may give a clue as to the protein's function.

**Grant:** 2R01GM039583-15  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** CARLSON, RUSSELL W PHD  
**Title:** Rhizobial Lipopolysaccharides Essential for Infection  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 1988/06/01-2006/03/31

DESCRIPTION (provided by applicant): Rhizobia are Gram-negative soil bacteria that form nitrogen-fixing symbioses with legumes. A specific rhizobial species recognizes and infects a specific host plant. Rhizobial cell surface lipopolysaccharides (LPSs) are important for endocytotic invasion of host root nodule cells and differentiation into nitrogen-fixing bacteroids within a host-derived intracellular compartment known as the symbiosome. The symbiosome membrane is derived from the Golgi and endoplasmic reticulum of the host cell and is both acidic and low in O<sub>2</sub>. Rhizobial symbionts are analogous to animal bacterial pathogens that survive in phagosome-derived intracellular compartments and cause chronic infections; e.g. *Brucella*. The LPSs from members of the Rhizobiaceae have a very long chain lipid-A fatty acid, 27-OHC28:0, (as does the pathogen, *Brucella abortus*). Also, LPSs from *R. etli* (Re) (a bean symbiont) and from *R. leguminosarum* bv. *viciae* (Rlv) (a pea symbiont) have unique lipid-A, and core oligosaccharide; e.g. they are devoid of phosphate and contain galacturonic acid. During symbiosis, modifications are made to the O-chain polysaccharide (e.g. methylation) and lipid-A. In the case of Rlv, both LPS and the entire bacterium become hydrophobic. This increase in hydrophobicity is due primarily to a doubling of the lipid-A 27-OHC28:0 moiety. It is proposed that the LPS O-chain modifications are required for adherence of the bacterium to the host membrane, and that the 27-OHC28:0 moiety is required to maintain membrane stability during endocytosis and symbiosome formation. The aims of this proposal are to structurally characterize the O-chain modifications, and to determine the functions of the Re and Rlv unique structural features with regard to both symbiosis and LPS biosynthesis. This will be accomplished through the creation and analysis of Re and Rlv mutants that are specifically altered in these unique structural features (e.g. defective in 27-OHC28:0 incorporation), and by the isolation and characterization of LPS fragments that bind to specific monoclonal antibodies whose LPS epitopes change during symbiosis.

**Grant:** 2R01GM039736-14  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** MANSON, MICHAEL D PHD  
**Title:** Chemoreception and Signal Amplification  
**Institution:** TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX  
**Project Period:** 1988/04/01-2006/02/28

Bacterial chemotaxis is a virulence factor for pathogenic bacteria and is a model for recognition of, and response to chemicals in unicellular and multicellular organisms. The proposed research extends work done under the previous grant and expands it into new areas. Our study of the interaction between periplasmic maltose-binding protein (MBP) and the Tar chemoreceptor will focus on structural studies and modeling. The question of whether covalent adaptation, like transmembrane signaling, is asymmetric between the two subunits of the Tar homodimer will be addressed, as will the structural basis for the inability of Tar from Salmonella to mediate maltose taxis. Hypotheses about the role of tryptophan residues at the borders of transmembrane helix II of Tar will be tested with site-directed mutagenesis. We will also analyze interaction of the dipeptide-binding protein (DppA) and its cognate receptor, Tap, in genetic studies designed to define the mechanisms by which binding-protein/transmembrane-receptor pairs function in chemotaxis. We have created a chimera between the NarX sensor kinase and Tar that is a repellent receptor for nitrate/nitrite. We will generate the reciprocal Tar/NarX fusion to determine how its kinase activity is regulated by Tar attractants and repellents. We will also create reciprocal chimeras between Tar and other sensor kinases, such as the osmosensor EnvZ of E. coli and Salmonella and the cell-density sensor SasS of Myxococcus xanthus. These hybrids will provide further information on mechanisms of transmembrane signaling, allow analysis of the ligand-recognition properties of the sensor kinases, and produce sensors that can be used to engineer bacteria to be detectors of environmental chemicals. Finally, we are interested in dynamic complexes that form between membrane receptors and soluble signaling proteins. Phosphorylation systems reconstituted in vitro will combine multiple receptors, CheW coupling factor, CheA kinase, and CheY response regulator to monitor receptor cross-talk. This issue will be addressed in vivo by comparing chemotaxis in cells in which different receptors are expressed sequentially versus simultaneously. The demonstration that fusions between CheZ and fluorescent proteins (GFP or YFP) retain CheZ function and subcellular localization suggests that these constructs can be used to probe the factors that affect localization of CheZ to the chemoreceptor patch, to assess the significance of CheZ localization in signal transduction, and to examine the sequence of events that leads to formation of the patch in growing and dividing wild-type cells and cell-division mutants. The supramolecular architecture of the receptor patch and its associated proteins will be examined using electron tomography.

**Grant:** 2R01GM039777-14  
**Program Director:** JONES, WARREN  
**Principal Investigator:** JULIN, DOUGLAS A PHD  
**Title:** Mechanism of the RecBCD Enzyme from E. coli  
**Institution:** UNIVERSITY OF MARYLAND COLLEGE PK COLLEGE PARK, MD  
CAMPUS  
**Project Period:** 1989/04/01-2006/03/31

DESCRIPTION (provided by applicant): The RecBCD enzyme acts in homologous DNA recombination in Escherichia coli and other bacteria. Homologous recombination is essential for the repair of some types of DNA damage and is an important part of the DNA replication process. Bacteria with mutations in the recB or recC genes are deficient in their ability to carry out homologous recombination and they have low viability. That is, a large fraction of the cells are inviable after cell division as a result of the inability to repair chromosome breaks that arise during DNA replication. Homologous recombination is also important in DNA repair and replication in higher organisms including humans. Defects in these processes have significant negative health implications, as they can be a contributing factor in the development of diseases including cancer. RecBCD is also quite interesting as a multisubunit enzyme machine. It is a multifunctional enzyme that unwinds and degrades linear DNA as an ATP-dependent exonuclease. The enzyme activity is regulated by a specific DNA sequence that has been called Chi (5'-GCTGGTGG). An encounter with Chi affects the rate and strand specificity of the nuclease reaction. All of these phenomena are critically dependent on the reaction conditions, especially the ATP and magnesium ion concentrations. The principal investigator has studied the enzyme by dissecting it into its subunits and protein domains and characterized the activities of those component parts. This proposal describes enzymological experiments to study how the enzyme activities and structure are affected by ligands including magnesium ion, DNA, and ATP, to learn more about the regulation of the enzyme activity that happens at the Chi sequence. He will also study in more detail the structure of a small domain of the RecB subunit that has the nuclease active site, how its activity is affected by the presence of the other proteins, and comparative experiments with another nuclease that has a similar active site sequence.

**Grant:** 2R01GM040313-13  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** ESCALANTE-SEMERENA, JORGE C PHD  
**Title:** B12 Biosynthesis And Anaerobi Metabolism In Salmonella  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 1988/09/01-2005/11/30

DESCRIPTION (provided by applicant): Understanding the integration of metabolic pathways will be one of the challenges for biologists in the post-genomics era. To meet this challenge, cell physiologists will rely on solid knowledge of the biochemistry underpinning the metabolic pathways in the cell. The study of major metabolic pathways like the one dedicated to the synthesis of adenosylcobalamin (coenzyme B12) offers an opportunity to reveal the strategies used by the cell to manufacture the most structurally complex coenzyme whose steady intracellular level is very low, and to learn how this major pathway is integrated with other metabolic processes in the cell. De novo synthesis of coenzyme B12 is performed only by procaryotes, but it is an essential nutrient for humans and animals in general. A diet devoid of cobalamin leads to a condition known as pernicious anemia. The inability of the human cell to convert the vitamin form to its coenzymic form results in severe health problems. Conversion of the vitamin to the coenzymic form requires the attachment of the upper ligand 5'-deoxyadenosine, and this reaction is catalyzed by an adenosyltransferase enzyme. One of the objectives of the proposed work is to further our understanding of how a bacterial adenosyltransferase enzyme works. This knowledge will serve as the foundation for future work on the human enzyme aimed at solving the problem of the lack or lower activity of this enzyme. We are also studying the enzyme that catalyzes a reaction critical to the use of precursors present in the environment and to de novo coenzyme B12 biosynthesis. A complete understanding of how this enzyme works will facilitate the design of drugs that could block its activity, thus preventing salvaging and de novo synthesis of coenzyme B12. Gaps of knowledge remain regarding many aspects of the coenzyme B12 biosynthetic pathway. We take a multifaceted approach to fill these gaps, and work together with structural biologists and chemists to bring our understanding of these processes to a level not afforded by single-discipline . approaches.



**Grant:** 2R01GM040941-14  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** BAUER, CARL E  
**Title:** Prokaryotic Gene Regulation By Light and Oxygen  
**Institution:** INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN  
**Project Period:** 1989/12/01-2005/11/30

DESCRIPTION (provided by applicant): Alterations in oxygen tension are known to affect gene expression in all cell types. In bacterial systems, oxygen affects expression of a variety of genes involved in aerobic versus anaerobic energy generation such as nitrate reductase and cytochrome oxidase. The presence of oxygen reactive species, also affects expression of genes involved in defense against oxidative damage. A variety of additional metabolic processes such as carbon fixation, nitrogen fixation, and anoxygenic photosynthesis are also regulated in response to the presence or absence of oxygen. In yeast and algal cells, oxygen is known to affect transcription of oxidative defense genes as well as enzymes involved glycolysis. In mammalian cells, a growing number of genes are known to be oxygen regulated such as those coding for growth factors such as erythropoietin and vascular endothelial growth factors that are key regulators for the synthesis of new capillary sprouts from preexisting vessels. Besides physiological roles, these growth factors are also involved in disease processes such as the stimulation of capillary formation during tumor growth. This proposal is centered on elucidating molecular details of how oxygen affects gene expression in bacteria. As a model system, we study oxygen regulated gene expression in *Rhodobacter capsulatus* which is closely related to the mitochondrial lineage. This species is capable of growth in a variety of energy generating modes including aerobic respiration, anaerobic fermentation and photosynthesis. The expression of genes involved in each of these processes is known to be affected by alterations in oxygen tension and well as by variations in light intensity. As such, this organism offers itself as an important model system to the study oxygen and light regulation of gene expression in a number of biological systems.

**Grant:** 2R01GM043854-13  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** RYCHNOVSKY, SCOTT D  
**Title:** Synthesis of Macrolide and Oxygenated Natural Products  
**Institution:** UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA  
**Project Period:** 1990/04/01-2006/05/31

DESCRIPTION (provided by applicant): Synthetic organic chemistry makes new compounds available for all fields of science, and synthetic chemists develop the tools to assemble chemical compounds more effectively. This proposal focuses on syntheses in four different classes of natural products. Synthesis of candidin and rimocidin, both polyene macrolides with potent antifungal properties, will be completed. The principle remaining challenge is attachment of the B-mycosamine sugar to the aglycones, and several new strategies have been developed to address this problem. The second compound of interest is SCH 351448, a complex macrolide that is the first small molecule activator of the LDL receptor promoter. This activity suggests a new therapeutic approach to controlling serum cholesterol levels. It will be synthesized using the 4-acetoxy-1,3-dioxane synthons developed in the previous grant period. Apicularen A is the third target. It is a very potent inhibitor of human cancer cell lines. Its preparation features a cyanohydrin acetonide coupling and a formal benzylic anion addition to an oxocarbenium ion. The final natural product is amphidinol 3. It has potent antifungal activity, and its structural assignment is provocative and worth investigating. Our proposed synthesis features a number of unusual convergent coupling strategies. The synthetic targets are challenging and interesting, and the new methods developed in these projects will be applicable to many other structures of contemporary interest.

**Grant:** 2R01GM044974-10A1  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** MERZ, KENNETH M  
**Title:** Mettalloenzyme Structure/Function  
**Institution:** PENNSYLVANIA STATE UNIVERSITY-UNIV UNIVERSITY PARK, PA  
PARK  
**Project Period:** 1991/04/01-2006/03/31

The long-term goal of this research is to understand, at the molecular level, the catalytic mechanism and inhibition of beta-lactamases and through this understanding facilitate the development of small-molecule therapeutics. Bacterial resistance to beta-lactam antibiotics has emerged over the past decade as a major health concern. Beta-lactam antibiotics kill bacteria by preventing the complete synthesis of the bacterial cell wall leading to a defective cell wall, which ruptures under the high internal pressure of the cell. Bacteria have developed antibiotic-resistance strategies in three major ways: production of hydrolytic enzymes known as beta-lactamases, changes in the permeability of the cell membrane, and alterations of the target enzymes. Among these mechanisms, beta-lactamase production, relentlessly fueled by natural selection, is generally considered as the primary route of resistance to beta-lactam antibiotics. Significantly, these enzymes can be chromosome or plasmid encoded and are secreted into the periplasmic space of Gram-negative bacteria or into the outer medium by Gram-positive bacteria, which facilitates the spread of beta-lactam resistance. The emergence of anti-beta-lactam activity also has a tremendous social and financial impact because of the continuous need to discover novel antibiotics. The tools that will be used to reach the long-term goal are those of theoretical chemistry, medicinal chemistry and biochemistry. The primary enzymes that will be studied are the beta-lactamases from *B. cereus* and *B. Fragilis*. With the aid of these tools the nature and energetics of beta-lactamase-substrate interactions, beta-lactamase-inhibitor interactions and reactions catalyzed by these beta-lactamases will be examined. The insights obtained into these processes will have a major impact on human health by facilitating the design of new drugs that will eliminate at least one bacterial mechanism for anti-beta-lactam activity, which will in turn increase the lifetime of existing antibiotics.

**Grant:** 2R01GM047369-09  
**Program Director:** JONES, WARREN  
**Principal Investigator:** LYNN, DAVID G  
**Title:** Chemical & Genetic Approaches to Define Cell Activation  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 1992/04/01-2005/12/31

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

**Grant:** 2R01GM047601-10  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** THOMPSON, LYNMARIE K PHD CHEMISTRY  
**Title:** Mechanisms of Transmembrane Signaling  
**Institution:** UNIVERSITY OF MASSACHUSETTS AMHERST, MA  
AMHERST  
**Project Period:** 1992/05/01-2006/06/30

DESCRIPTION (provided by applicant): Membrane proteins account for ~30% of proteins encoded by various genomes and play critical roles in biological organisms. However, structural biology of membrane proteins lags far behind that of their soluble counterparts, which hampers efforts to understand their mechanisms and to fully exploit them as drug targets. The bacterial chemoreceptor family is an ideal system for investigating the molecular mechanism of transmembrane signaling, a fundamental process mediated by membrane proteins. During the current funding period, we have established a site-directed solid-state NMR distance measurement approach capable of measuring local structure in large membrane proteins, and have made the first distance measurements in the intact, membrane-bound Ser receptor with sufficient resolution to measure the subtle changes thought to transmit the signal. Aims 1-3 of the this proposal will use site-directed solid-state NMR to measure interhelical distances in the periplasmic, transmembrane, and cytoplasmic domains of the intact, membrane-bound Ser receptor to map the intra- and inter-subunit conformational changes induced by ligand-binding and receptor methylation. These experiments will also refine the structural model of the transmembrane helices, measure the secondary structure of the critical linker region (Aim4), test proposed dimer-dimer contacts in the cytoplasmic domain to elucidate the structure of receptor clusters, and measure the orientation of amides throughout the receptor to test the overall structural model (Aim 5). The overall goal is to develop and use an integrated solid-state NMR/biochemical approach to obtain high-resolution information on the intact, membrane-bound Ser receptor, which is unavailable by other methods, to reveal the structural basis of the mechanism of transmembrane signaling. These integrated approaches will be applicable to studies of structure & function in other important membrane protein systems.

**Grant:** 2R01GM047645-09  
**Program Director:** FLICKER, PAULA F.  
**Principal Investigator:** SATTERLEE, JAMES D PHD  
CHEMISTRY:INORGANIC  
**Title:** Structure and Dynamics of Heme Protein Active Sites  
**Institution:** WASHINGTON STATE UNIVERSITY PULLMAN, WA  
**Project Period:** 1992/05/01-2006/07/31

DESCRIPTION (provided by applicant): This proposal involves characterizing the structures and chemistry of two very different types of heme proteins. The first of these is yeast cytochrome c peroxidase (CcP), which occurs naturally in yeast mitochondria and is a prototypical peroxidase. It is a -34KD ferriheme enzyme whose cellular function is to use reducing equivalents from its natural redox partner, cytochrome c (cytc), to decompose hydrogen peroxide. In this role it acts as a cytotoxic protective agent, participates in long-distance electron transfer and may also be important for oxidative stress signaling. The second group of proteins that we plan to study are a group of heme-based biological oxygen sensors. These include the FixLs from *Bradyrhizobium japonicum* (BjFixL) and *Sinorhizobium meliloti* (SmFixL) and the Direct Oxygen Sensor protein from *E. coli* (EcDos). The FixL proteins regulate expression of the *nif* and *fix* operons in their respective bacteria, which, in turn, control the biosynthesis of all proteins needed for nitrogen fixation. The EcDos protein is thought to participate in the aerobic/anaerobic switch in *E. coli*. All three of these proteins contain a central domain structure consisting of a PAS-heme binding domain (sensing domain) linked to a catalytic domain (kinase for the FixLs; phosphodiesterase for EcDos). While these three sensors are of bacterial origin, it has recently been noted that molecular events triggering human renal fibrosis, resulting from hypoxia involves a protein with heme-based oxygen sensing linked to protein kinase catalysis, similar to the FixLs (Norman, J. T., Clark, J. M., and Garcia, P. L., "Hypoxia Promotes Fibrogenesis in Human Renal Fibroblasts," (2000) *Kidney Int.*, 58, 2351-2366). All four of the heme proteins that we intend to study (CcP, FixLs, EcDos) are already being expressed and studied in our laboratory. The goals for both protein types are the same. We propose an integrated effort to study their function, structure and dynamics. The goal is to elucidate how they function on a molecular basis, and what structural features are critical to that function. We shall proceed using modern protein engineering methods combined with x-ray crystallography, kinetics, photothermal methods, equilibrium dynamics methods and NMR spectroscopy.

**Grant:** 2R01GM047823-09  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** HENKIN, TINA M  
**Title:** Regulation of *Bacillus subtilis* tRNA synthetase genes  
**Institution:** OHIO STATE UNIVERSITY COLUMBIUS, OH  
**Project Period:** 1993/12/01-2006/12/31

DESCRIPTION (provided by applicant): The *Bacillus subtilis* tyrS gene is a member of a large group of aminoacyl-tRNA synthetase, amino acid biosynthesis and transporter genes, designated the T box family, that are regulated by a unique transcription termination control system. Expression of each gene is dependent on interaction of the leader region of the transcript with a specific uncharged tRNA. This interaction promotes formation of an antiterminator structure, preventing premature termination of transcription. We have identified over 250 transcriptional units, primarily from Gram-positive bacteria, with leader regions exhibiting the conserved elements characteristic of members of this family. Several important pathogens, including *Bacillus anthracis*, *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Mycobacterium*, are represented in this group. Since most of the regulated genes encode essential proteins, this system represents a potential target for antibiotic development. Expression of each gene in the family is dependent on pairing of the anticodon of the inducer tRNA with a single codon, the "specifier sequence," in the leader, and on pairing of acceptor end of the tRNA with a bulged region of the antiterminator; these pairings are necessary but not sufficient for efficient antitermination. Phylogenetic analysis of the leaders has revealed a number of elements the structure of which can be predicted based on similarity to other RNAs. Novel variations on the arrangement of conserved leader elements has also been uncovered; some of these variations are suggestive of variability in the molecular mechanism of antitermination. The next project period will focus on using the phylogenetic data as a basis for efforts to uncover additional structural and mechanistic features of the system. The approaches used will include computational analyses of our extensive aligned leader RNA and tRNA database, and structural studies of leader elements in parallel to our successful analysis of the antiterminator domain. Genetic and biochemical approaches will be directed to the refinement of the required leader and tRNA elements, and for identification of possible additional factors.

**Grant:** 2R01GM047909-08  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** KRANZ, ROBERT G  
**Title:** Cytochrome c biogenesis  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 1994/09/01-2007/02/28

DESCRIPTION (provided by applicant): Cytochromes are heme proteins essential for aerobic and anaerobic electron transport in most organisms. While the structures and functions of many of these proteins have been studied for over fifty years, only relatively recently has it become clear that cytochromes often require assembly factors. Such assembly factors are defective in certain human disorders, and essential for growth in many pathogens. Biogenesis of the c-type cytochromes requires a large number of factors and can proceed by any one of three systems. Prokaryotes, plant mitochondria, and chloroplasts use either system I or II, which are each predicted to require dedicated mechanisms for heme delivery and apocytochrome thiolreduction. In system III, which has specifically evolved in the mitochondria of fungi, invertebrates, and vertebrates, a pivotal role is played by a single enzyme called cytochrome c heme lyase in the mitochondrial intermembrane space. Cytochrome c biogenesis requires nine dedicated, integral membrane proteins for System I and at least three for System II. The goals of this proposal are to understand the molecular mechanisms by which Systems I and II operate using three model proteobacteria, *Rhodobacter capsulatus* (System I), *Bordetella pertussis* (System II), and *Escherichia coli* (System I and recombinant System II). Specifically, aims are to (1) identify and characterize novel System II genes in *B. pertussis*; (2) reconstitute in vitro biogenesis using System I and System II; (3) determine the substrate recognition requirements (ie. apocytochrome and heme) and the physiological conditions essential for System I and II; (4) carry out a functional analysis of key proteins involved in System I and II.



**Grant:** 2R01GM048147-10  
**Program Director:** GREENBERG, JUDITH H.  
**Principal Investigator:** GOBER, JAMES W PHD  
**Title:** Generation of Asymmetry During Caulobacter Development  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 1992/08/01-2005/11/30

**DESCRIPTION:** The bacterium *Caulobacter crescentus* undergoes a simple cellular differentiation within each cell cycle. Cell division generates two distinct cell types: a motile swarmer cell and a sessile stalked cell. These two cell types differ with respect to their relative programs of gene expression and DNA replication. The generation of asymmetry upon cell division is a fundamental aspect of development in diverse organisms, including *Drosophila*, nematodes and fungi. In progeny stalked cells, the components of the polar flagellum are expressed under cell cycle control and assembled at the pole of the predivisive cell that lies opposite the stalk. Two well-defined developmental checkpoints regulate flagellar morphogenesis. First, the transcription of early flagellar genes is triggered by an unknown cell cycle event that is linked to the initiation of DNA replication. Later in the cell cycle, the transcription of late flagellar genes is activated by a cell division event and the assembly of early flagellar structures. We propose that these events result in the compartmentalized transcription of both late and early flagellar genes. The overall objectives of this proposal are to define the mechanisms that couple early flagellar assembly to cell-type-specific expression of late flagellar genes. The proposed experiments, for the most part, focus on the activation of the response regulator transcription factor, FlbD, and its role in regulating temporal and spatial transcription. In addition, in order to explore cell-type specific translation, we describe experiments designed to identify the factors that regulate FlbT and the translation of flagellin genes. Finally, we propose to identify genes outside of the flagellar regulon that are also controlled by FlbD. In order to accomplish these goals the biochemical properties of constitutive alleles of FlbD will be characterized and their effect on temporal and spatial transcription will be analyzed. In addition, the FlbD kinase will be identified. The role of the novel regulator FliX in coupling flagellum morphogenesis to gene expression will be determined by dissecting the mechanisms underlying FliX-mediated repression and activation of FlbD, and determining how the assembly of a flagellar structure regulates FlbX activity. In order, to investigate the role of posttranscriptional regulation of cell-type-specific gene expression, proteins that regulate FlbT activity will be identified. Finally, DNA microarray gene expression assays will be conducted in order to identify critical cell cycle-related genes that are regulated by FlbD. The role of these genes in cell cycle progression will then be analyzed.

**Grant:** 2R01GM048220-09

**Program Director:** ANDERSON, JAMES J.

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

**Title:** Control of Sigma B Activity in B subtilis

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

**Project Period:** 1992/09/30-2006/04/30

**DESCRIPTION:** (provided by applicant): In response to environmental stress, sigma B, a transcriptional regulator of *B. subtilis*, is released from an inhibitory association with an anti-sigma B protein (RsbW) to activate expression of the bacterium's general stress regulon. The stress-generated signal that activates sigma B is unknown; however, recent evidence implicates the bacterium's ribosome and a small GTP binding protein (Obg) in this process. The proposal seeks to determine the roles of the ribosome and Obg in the stress induced activation of sigma B. Both Obg and the non-essential ribosome protein L11 are needed for environmental stress to activate sigma B. We will perform directed and random alterations of the coding sequences for each of these proteins to identify regions or activities that modify the inducibility of sigma B and determine how these changes influence each of these proteins' other known functions. The analyses should identify regions of Obg and L11 that are important for their biochemical activities and suggest which of these properties are required for sigma B induction. Several components of the stress activation cascade (RsbR, S and T) have been observed to cofractionate with ribosomes. This putative association will be examined, using velocity centrifugation and gel filtration analyses under conditions that are likely to cause partial or total dissociations. The specific ribosome fraction components and Rsb proteins that are involved in the associations will be identified. The types of complexes found and the identities of proteins directly involved could give clues as to the role of the association in stress signaling and sigma B induction. Finally, a detailed mutational analysis will be undertaken of rsbT, the most upstream positive regulator in the sigma B stress induction pathway and the gene whose product is the most likely to be directly influenced by stress signaling. It is anticipated that the changes in rsbT and their resulting phenotypes will provide a test of RsbT's proposed activities as well as identify sites where stress directed signals alter RsbT activity. This work will not only explore the fundamental biological question of how cells recognize and react to hostile environments, but, given the presence of sigma B and its principal regulators in the human pathogens *Staphylococcus aureus*, *Listeria monocytogenes* and *Mycobacterium tuberculosis*, it may have practical applications, identifying weaknesses in these pathogens' stress adaptation responses to host defenses.

**Grant:** 2R01GM048445-10  
**Program Director:** JONES, WARREN  
**Principal Investigator:** DIGATE, RUSSELL J PHD  
**Title:** Mutational Analysis of E coli DNA Topoisomerase III  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 1993/01/01-2006/02/28

DESCRIPTION (Provided by applicant): DNA topoisomerases are enzymes that alter the linking number of DNA and, as such, are responsible for topological inter- and intra-conversions within DNA molecules. These conversions include the knotting or unknotting of DNA, the supercoiling or relaxation of DNA (an intra-molecular conversion) and the catenation (or decatenation) of DNA (an inter-molecular conversion). Topoisomerases can be classified into two major categories, type I and type II. The type I enzymes can be further subdivided into type IA prokaryotic DNA topoisomerase I (Topo I) and III (Topo III), and eukaryotic DNA topoisomerase III (Topo III) and type IB (eukaryotic DNA topoisomerase I and bacteriophage recombinases). It has been shown that Topo III-like activities appear to function in homologous pathways in both prokaryotes and eukaryotes. In particular, it is becoming increasingly evident that Topo III-like enzymes interact with DNA helicases and act in the suppression of recombination in both yeast and E. coli. Topo III of E. coli is extremely amenable to both biochemical and genetic characterization; therefore, the characterization of E. coli Topo III has been very useful to establishing the role of these enzymes in the DNA metabolism of both prokaryotes and eukaryotes. The specific aims of this grant are: 1. Further identify residues involved in E. coli Topo III-mediated catalysis and elucidate the mechanism of type IA topoisomerase-mediated catalysis 2. Elucidate the role of E. coli Topo III in recombination 3. Identify and examine the role of topoisomerase III interactions with other cellular proteins, in particular, DNA helicases.

**Grant:** 2R01GM048746-10  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** CHRISTIE, PETER J PHD  
**Title:** DNA Translocation Across the Agrobacterium Envelope  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX  
HOUSTON  
**Project Period:** 1993/01/01-2005/12/31

DESCRIPTION (provided by applicant): Macromolecular translocation across prokaryotic and eukaryotic membranes is a major area of biomedical interest. In recent years, secretion systems ancestrally-related to bacterial flagellar and conjugation systems have been shown to play important roles in pathogenesis by translocating effector molecules to the eukaryotic cell cytosol during the course of infection. These systems are now designated as types III and IV secretion pathways, respectively. The focus of work in this laboratory is the type IV transfer system used by *A. tumefaciens* to deliver oncogenic T-DNA to susceptible plant cells. The T-DNA transfer system is an excellent model for detailed mechanistic studies of type IV secretion. Recent work has shown that the T-DNA transfer system is exceptionally versatile both in terms of substrate selection and target cell recognition. In addition to the T-DNA secretion substrate, this system can export other DNA substrates as well as effector proteins independently of DNA. Furthermore, this system can translocate substrates to other bacteria and to a wide variety of eukaryotic cell types, including those of plants, fungi, and humans. The overall goals of work in this laboratory are to: i) define the assembly pathway for this type IV transfer system, ii) characterize its architectural arrangement, iii) elucidate the reaction mechanisms underlying substrate processing and delivery to the secretion channel, and iv) define the route of translocation across the Gram-negative bacterial envelope. It is now established that substrate transfer requires a pilus for mediating cell-cell contacts and a channel for translocation across the bacterial cell envelope. We will use a combination of molecular, genetic, and biochemical approaches in the following specific aims. First, we will explore structure - function relationships of the inner membrane VirB11 ATPase to define its role in transporter biogenesis and substrate translocation; for these studies we will capitalize on our assemblage of a large collection of altered-function mutants and a crystal structure of a VirB11 homolog. Second, we will identify steps in the substrate delivery pathway, focusing on the model VirE2 substrate and the VirE1 secretion chaperone. Finally, we will characterize critical steps in the assembly pathway of this transfer system; these studies will identify specific contacts involving three proteins essential for transporter assembly and structural integrity, the polytopic inner membrane protein VirB6 and two outer membrane proteins, VirB7 lipoprotein and VirB9.

**Grant:** 2R01GM048804-09A1  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** BECHHOFFER, DAVID H PHD  
**Title:** Initiation of mRNA decay in *Bacillus subtilis*  
**Institution:** MOUNT SINAI SCHOOL OF MEDICINE OF NEW YORK, NY  
NYU  
**Project Period:** 1993/01/01-2006/06/30

DESCRIPTION: (provided by applicant): Our laboratory seeks to understand the control of mRNA decay in *Bacillus subtilis*. For the Gram-positive bacteria, much needs to be learned about the features of an mRNA that determine its half-life, the ribonucleolytic reactions involved in mRNA decay, the genes that encode ribonuc the regulation of their expression. Since the *B. subtilis* genome does not have sequence homologues for several of the major ribonuclease genes of *Escherichia coli*, it is altogether uncertain whether models for mRNA decay based on the well-studied *E. coli* system will pertain to *B. subtilis*. Small RNA molecules will be used to probe three facets of mRNA turnover in *B. subtilis*: 1) the entry site for 3' exonucleolytic degradation; 2) endonucleolytic cleavage that initiates decay; and 3) role of the 5' end in determining mRNA half-life. These small RNA molecules are designed such that analysis of their decay will begin to clarify how mRNA turnover is achieved. To identify additional ribonuclease genes (three have been cloned thus far), biochemical experiments are proposed to isolate several proteins predicted to be involved in mRNA decay, i.e., at least one additional 3'-to-5' exoribonuclease, a putative endoribonuclease, and poly(A) polymerase. Once the identities of these proteins are known, the genes encoding them will be disrupted in order to study the effects on mRNA decay. To develop our understanding of ribonuclease function in the Gram-positive bacteria, we will study the function of Bs-RNase III, a narrow-specificity endoribonuclease that has been shown to be essential in *B. subtilis*. Genetic means will be employed in an effort to understand the role of Bs-RNase III that is critical for viability. The basis for control of Bs-RNase III activity in the cell will be investigated, providing the first look at ribonuclease gene regulation in *B. subtilis*.

**Grant:** 2R01GM050151-09  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** SNIDER, BARRY B PHD CHEMISTRY, OTHER  
**Title:** SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS  
**Institution:** BRANDEIS UNIVERSITY WALTHAM, MA  
**Project Period:** 1997/12/01-2005/11/30

This proposal consists of seven unrelated projects designed to prepare structurally novel, biologically active natural products of potential interest as drugs and to explore new synthetic methods of general interest. (1) The first synthesis of the novel pyrroloquinolines martinellie acid and martinelline will be completed. These alkaloids inhibit several G-protein coupled receptors. (2) New methods have been developed and used for the first syntheses of fumiquinazolines A, B, G, and I and asperlicin. This project will be completed by the synthesis of the more highly oxidized fumiquinazolines C, D, E, and H, fiscalins A and B, and citreosindole. (3) The novel diterpene antibiotic guanacastepene that appears to function by disruption of membranes will be prepared using a novel route to the hydroazulene ring system. This chemistry will be used for a short synthesis of the CD ring system of vitamin D. (4) Haterumalide B and haterumalide NA (oocydin A) are novel macrolides isolated from a sponge, ascidian and phytopathogen that are cytotoxic and show selective toxicity to breast cancer cells. An efficient route to these compounds using a Stille coupling of an allylic bromide or acetate with a vinylstannane to construct the skipped chlorodiene unit will be developed. (5) Cytoskyrin A, graciliformin, and rugulosin are unusual bisanthraquinoid natural products that have potent biological activity and structural novelty. They will be synthesized by a biogenetic route using a phenyldimethylsilyl group as a latent hydroxy group. (6) Phloeodictine A and A1-A7 are a family of guanidine containing amidinium salt natural products that are cytotoxic and antibacterial. In model studies, a general new route to these compounds has been developed by adding Grignard reagents to the acyl group of an N-acylamidine and then alkylating the amidine. This will be extended to the natural products and analogues. (7) Mn(III)-based oxidative cyclization of Meldrum's acid derivatives occurs almost instantaneously at 25 degrees celcius to give predominantly cyclohexene products. The mechanism and synthetic utility of these cyclizations will be developed.

**Grant:** 2R01GM050441-09  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** BILLIAR, TIMOTHY R  
**Title:** Post-Traumatic Sepsis: Regulation of LPS Binding Protein  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA  
PITTSBURGH  
**Project Period:** 1994/01/01-2006/03/31

**DESCRIPTION** (provided by applicant) The liver is a primary response organ in post-operative infections. Hepatocytes (HC), the primary cell type in the liver, take up and metabolize microbial products, respond directly to lipopolysaccharide (LPS), and release proteins into the circulation that regulate host-microbe interactions. In contrast to leukocytes and sepsis, relatively little is known about the anti-microbial responses of HC. We have previously shown that HC express both CD14 and LBP, and that the expression of both of these proteins is increased in endotoxemic rats. More recently, we have shown that the endotoxemic liver expresses increased levels of TLR2, and that HC upregulate TLR2 in response to pro-inflammatory cytokines. In HC, functional TLR4 appears to be required both for stimulation with LPS, as well as for LPS-mediated desensitization. We suggest that LPS may desensitize HC by modulating signal transduction proteins common to TLR2, TLR4, and IL-IR1. We hypothesize that HC express microbial recognition proteins for three specialized purposes. First, HC possess the capacity to respond directly to microbial products, in a CD 14- and TLR-dependent manner to permit a rapid response to serious infections. However, these responses are downregulated by the microbial products themselves and/or by cytokines released subsequent to stimulation with these microbial products. Second, surface CD14 and TLR on HC participate in the clearance of microbial products. Third, HC regulate the systemic response to infection through the release of soluble CD14. We will pursue these hypotheses in three interrelated Aims: 1) to determine the signaling function of microbial recognition systems in HC; 2) to determine the role of HC LPS recognition molecules in LPS clearance and the systemic response; and 3) to determine the mechanism of LPS-mediated desensitization of HC. We will take advantage of LBP, CD14, TLR2, and TLR4 null mice, using both in vitro and in vivo systems to fully assess the roles of the LBP/CD14/TLR pathway in the liver. Insights gained from these studies should significantly enhance our understanding of the earliest events in host-microbe interactions in surgical sepsis.

**Grant:** 2R01GM050870-10  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** QURESHI, NILOFER PHD  
**Title:** MECHANISMS INVOLVED IN THE SEPTIC SHOCK SYNDROME  
**Institution:** UNIVERSITY OF MISSOURI KANSAS CITY KANSAS CITY, MO  
**Project Period:** 1994/05/15-2006/04/30

DESCRIPTION (provided by applicant): Sepsis afflicts approximately 500,000 Americans per year with an associated mortality of approximately 35-65 percent. The lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacteria, is one of the initiating stimulants in an inflammatory cascade that has been referred to as the "Systemic Inflammatory Response Syndrome." During the past seven years, we have analyzed the interactions of LPS with cells of the inflammatory process that can result in mortality in Gram-negative sepsis. The long-range goal of this research program is to understand the molecular mechanisms of pathogenesis of septic shock and to identify potential therapeutic intervention strategies to reduce morbidity and mortality resulting from this collection of diseases. The goal of this application is to examine a novel pathway by which LPS can be internalized by mouse macrophages, and following interaction with cytoplasmic organelles, trigger the production of proinflammatory cytokines and other mediators of inflammation. The central hypothesis of this research project is that LPS, acting at least in part through interactions with heat shock proteins, interacts with the cytoplasmic proteasome of macrophages resulting in their proteolytic activation and resultant generation of proinflammatory cytokines. It is further hypothesized that interference with this pathway by appropriate antagonist molecules will inhibit the production of proinflammatory cytokines and provide protection against LPS-induced septic shock. The following Specific Aims are proposed: 1. To complete the molecular characterization of specific LPS-binding proteins identified in cellular subfractions of macrophages. We will employ our newly designed photoreactive LPS probe to specifically label the murine membrane and cytosolic proteins with binding affinity for LPS, purify these crosslinked complexes by 2D-gel electrophoresis, and identify the major crosslinked proteins by MALDI/MS. 2. To define the functional role of the LPS binding proteins-proteasome/heat shock proteins. 3. To evaluate the relative contribution of the proteasome pathway to the pathogenesis of LPS-mediated septic shock. We anticipate that at the completion of these Specific Aims we will have a better understanding of the LPS-induced signal transduction pathways in macrophages. The successful completion of this research will contribute to the development of novel strategies for prevention and treatment of septic shock.



**Grant:** 2R01GM051215-09  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** ROSEMAN, SAUL PHD BIOLOGY  
NEC:BIOCHEMISTRY  
**Title:** Chitin catabolic cascade in *Vibrio cholerae*  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1994/08/01-2006/07/31

DESCRIPTION (provided by applicant): We are in our 8th cholerae pandemic, a disease that infects millions and kills over 100,000 humans annually. In one stage of its life cycle, the infective agent, *Vibrio cholerae*, lives as a commensal with copepods, a microcrustacean found in drinking water. Because the bacteria "burrow" into the cuticle, they escape the stomach acid barrier, to which free-living cells are sensitive. Thus, the chitinolytic properties of *V. cholerae* are directly relevant to human health and disease. In previous work with *Vibrio furnissii*, we found that chitin degradation involves a cascade, with at least three signalling systems and numerous genes and proteins, many of which were isolated. One of these was a sensor HK (histidine kinase) protein, which represents a breakthrough in this research. An in frame deletion of the sensor has a global effect on chitin utilization in that none of the characterized chitin cascade genes or processes were expressed. When the sequence of the *V. cholerae* genome became available, the predicted protein sequences of genes involved in chitin utilization that we had characterized in *V. furnissii* exhibited considerable identity to the corresponding ORFs in the *V. cholerae* genome. For example, the *V. furnissii* sensor is 84% identical and 93% similar to VC0622 over the full length of the predicted protein sequences. The sensor is homologous to the *E. coli* ArcB sensor, part of a two component signal transduction system. Over 21 ORFs in the *V. cholerae* genome are annotated as related to chitin catabolism (independent of GlcNAc catabolism). Of these, ten were characterized in *V. furnissii* but nothing is known about the remainder. We shall determine which genes are regulated by VC0622, using first an in frame deletion of VC0622. A second goal is to isolate and characterize the sensor HK protein from *V. cholerae*. Finally, we plan to identify and characterize the cognate HPt and RR protein(s) that interact with the sensor. The work will rely heavily on molecular biological and biochemical techniques, as well as genomics.

**Grant:** 2R01GM051310-09  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** RAETZ, CHRISTIAN R PHD  
BIOCHEMISTRY:BIOCHEM  
RY-UNSPEC  
**Title:** Biosynthesis and Function of Lipopolysaccharides  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 1994/09/01-2006/07/31

DESCRIPTION (provided by applicant): The outer leaflet of the outer membranes of Gram-negative bacteria is covered with a remarkable glycolipid known as lipopolysaccharide (LPS). In *Escherichia coli*, the lipid A anchor of LPS is a hexa-acylated disaccharide of glucosamine, bearing phosphate groups at the 1 and 4' positions. The minimal LPS required for growth of *E. coli* contains lipid A and Kdo sugars. The biosynthesis of lipid A is well characterized. Inhibition of any one of the enzymes catalyzing the first seven steps of the pathway in *E. coli* causes cell death. Lipid A is therefore an interesting target for designing new antibacterial agents. Emerging genomic sequences of diverse bacteria indicate that these enzymes are present in virtually all Gram-negative organisms. An unanticipated genomic surprise, however, is that orthologs of key enzymes for lipid A biosynthesis are also present in higher plants. Lipid A (endotoxin) is the active component of LPS that stimulates immune cells. During severe Gram-negative infections, the lipid A moiety of LPS can cause excessive activation of macrophages and endothelial cells. The resulting systemic over-production of certain inflammatory mediators and clotting factors damages small blood vessels. A full response to endotoxin leads to Gram-negative septic shock with multiple organ failure and death. An exciting potential therapeutic approach to this problem has emerged with the discovery that certain lipid A-like molecules, including some precursors, are endotoxin antagonists. The primary signaling receptor for lipid A is now known to be the TLR4 protein, which is distantly related to the IL-1 receptor. In earlier work, the P. I. discovered the nine constitutive enzymes for lipid A assembly in *E. coli*, and the genes encoding them. In the proposed work, the specific aims are: I) elucidation of the biosynthesis of lipid A variants containing four amide-linked fatty acids; II) re-engineering of the lipid A pathway in living *E. coli* cells; III) characterization of new PmrA and PhoP regulated enzymes that modify lipid A; IV) studies of cold shock and high Ca<sup>++</sup> induced lipid A modifications in *E. coli*; and V) analysis of lipid flip-flop and export in *E. coli*.

**Grant:** 2R01GM051426-09  
**Program Director:** WOLFE, PAUL B.  
**Principal Investigator:** SHAPIRO, LUCILLE PHD  
MICROBIOLOGY:MICROBIOLOGY-UNSPEC  
**Title:** Developmental Control of DNA Replication in Caulobacter  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 1994/08/01-2006/08/31

DESCRIPTION (provided by applicant): Our goal is to identify the signals and regulatory proteins that control DNA replication and chromosome segregation during the progression of a bacterial cell cycle. *Caulobacter crescentus* has a well-defined cell cycle which includes temporally and spatially constrained differentiation events. This bacterium is particularly well suited for an analysis of the control of chromosome replication and partition because cultures are easily synchronized and have a distinct G1-S transition, replication occurs in a specific cell type only once per cell cycle, and an asymmetric division yields separable progeny with different morphological features and replicative abilities. The annotated genome sequence of 3767 genes has been completed and we have generated DNA microarrays for the analysis of full genome transcription networks. In *Caulobacter*, members of the two-component signal transduction family of proteins play critical roles in many aspects of cell cycle control. In addition to serving as a repressor of replication initiation, the CtrA response regulator controls the expression of 30% of the 553 genes whose expression is under cell cycle control. Groups of genes involved in DNA replication that are expressed during the G1 -S transition are not among those controlled by CtrA. We will now identify the key regulatory factors that control these G1-S transition genes. At another level of regulation, the origin of replication is confined to the cell pole where the replisome assembles at the start of replication. We will determine the mechanisms by which the origin is dynamically localized to the cell pole, and the role of the SMC [Structural Maintenance of Chromosomes] protein in chromosome condensation and segregation. Finally, we have shown that newly replicated DNA remains in the hemi-methylated state until the end of the cell cycle. We will determine the role of the chromosome methylation state on cell cycle-regulated gene expression and chromosome segregation.

**Grant:** 2R01GM051554-09  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** SCHAEFER, JACOB PHD  
**Title:** Structure of Proteins in Cell Walls by REDOR NMR  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 1994/08/01-2006/07/31

DESCRIPTION (provided by applicant): We propose new solid-state rotational-echo double-resonance (REDOR) NMR experiments to determine in situ the mode of action of vancomycin and vancomycin analogues in actively dividing cells of *Staphylococcus aureus*. Both the vancomycins and the bacteria will be labeled with combinations of  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^2\text{H}$ , and  $^{19}\text{F}$ . Detection of the labels will use custom-built, high-efficiency 6-frequency transmission-line NMR probes. Three new types of REDOR experiments will provide site-specific detection of labels in cell walls of whole cells (both in suspension and aggregated in biofilms) with no interferences from cytoplasmic labels or from the natural-abundance background. REDOR experiments will also be performed on whole cells whose thick, outer layer of mature peptidoglycan has been removed. These protoplasts will be examined in various stages of reversion to normal bacteria, and so with varying amounts of attached nascent peptidoglycan. The vancomycin bound close to the exoface of the cytoplasmic membrane is therapeutically active. In addition to vancomycin, other peptide antibiotics including synthetic and natural magainins, nisin, and mersacidin will be used in REDOR experiments. Binding will be examined in whole cells, protoplasts, reverting protoplasts, multi-lamellar vesicles, and mechanically aligned bilayers on glass plates. The overall goal of the project is to use REDOR to define antibacterial modes of action thereby aiding the drug-discovery process aimed against anticipated lethal strains of *S. aureus* that are resistant to every presently known antibiotic.

**Grant:** 2R01GM051981-06A2  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** KAUR, PARJIT PHD  
**Title:** DOXORUBICIN TRANSPORT ATPase: MODEL FOR DRUG RESISTANCE  
**Institution:** GEORGIA STATE UNIVERSITY ATLANTA, GA  
**Project Period:** 1995/07/01-2006/01/31

**DESCRIPTION:** (provided by applicant) This project will investigate an ABC-type of transport ATPase, DrrAB, that confers resistance to doxorubicin and daunorubicin in the producer organism *Streptomyces peucetius*. DrrAB consists of two subunits DrrA and DrrB. Interestingly, DrrA bears homology to P-glycoprotein, a multidrug resistance protein, and to other members of the ABC family including CFTR and ABC 1. DrrAB and Pgp are also functionally similar: both confer doxorubicin resistance, DrrAB in the producer organism and Pgp in cancer cells. Because of the sequence, structural and functional similarity between DrrAB and Pgp, it is likely that they share a common ancestor. Hence, elucidation of the function of 'Drr' and the nature of the drug binding sites in 'Drr' will shed light on the mechanism of function of Pgp and on the evolution of multidrug resistance. Furthermore, DrrAB is ideal for understanding interaction between the membrane domain and the catalytic domain of ABC transporters. Preliminary experiments have shown that DrrA and DrrB are biochemically coupled; DrrA is required for the stability and maintenance of DrrB in the membrane and DrrB is required for the activity of DrrA. Experiments will be designed to test the hypothesis that DrrA forms a complex with DrrB and protects it from proteolysis before DrrB is targeted to the membrane. Experiments to study interaction between the two subunits will consist of both biochemical and genetic approaches including isolation of interaction-defective mutants and the second-site suppressors. These studies will have relevance in understanding targeting of membrane proteins and in elucidating the role played by the catalytic domains in stabilizing the membrane domains of multidomain or multisubunit proteins.

**Grant:** 2R01GM053017-05A1  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** YOTHER, JANET L PHD  
**Title:** MECHANISMS OF POLYSACCHARIDE SYNTHESIS BY *S. PNEUMONIAE*  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL  
BIRMINGHAM  
**Project Period:** 1995/07/01-2006/03/31

DESCRIPTION (provided by applicant): The polysaccharide capsules of *Streptococcus pneumoniae* represent the single most important virulence factor of this organism. Structurally and antigenically diverse, 90 serotypes have been recognized. Biosynthesis of these polysaccharides involves at least two distinct mechanisms. One, observed for the type 3 polysaccharide (-3)-beta-D-GlcUA-(1,4)-beta-D-Glc-(1-), involves a single processive enzyme (the type 3 synthase) that is responsible for generating both glycosidic linkages and for transporting the polymer out of the cell. The type 3 synthase is a member of a family of glycosyltransferases that includes the streptococcal and eukaryotic hyaluronan synthases, chitin synthases, and cellulose synthases. Our work with the type 3 synthase has shown that synthesis initiates on a lipid primer. Synthesis occurs by a processive mechanism under high substrate concentrations. Under low substrate concentrations, however, the synthase functions as a nonprocessive enzyme. When only a single substrate is present, chain termination and release of the polysaccharide result. Synthase stability in *S. pneumoniae* appears to be dependent on substrate levels, thus suggesting another means of controlling synthase function. The mechanism of synthesis of most other *S. pneumoniae* capsular polysaccharides is similar to that of some LPS O-antigens, involving initiation and transport of subunits across the membrane on a lipid carrier, followed by polymerization. Homologous proteins (CpsABCD) involved in this type of biosynthesis occur in *S. pneumoniae*, *Rhizobium*, other streptococci, and staphylococci. We have demonstrated an interaction among the *S. pneumoniae* proteins, and we hypothesize that they function to enhance polymerase activity. The goals of the proposed research are to characterize the *S. pneumoniae* polymerases and the factors that directly influence their functions. We will address these goals by: 1) identifying the primer for type 3 synthesis and reconstituting the system; 2) identifying functional domains/residues of the type 3 synthase; 3) characterizing synthase functions and polysaccharide release in vivo; and 4) characterizing functions common to the synthesis of non-type 3 capsular polysaccharides. The results of these studies will provide insights into mechanisms of polysaccharide synthesis that are shared among many prokaryotic and eukaryotic systems.

**Grant:** 2R01GM053439-06A1  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** KUDSK, KENNETH A MD  
**Title:** Enteral vs IV Feeding: Effect on Mucosal Immunity  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MEMPHIS, TN  
**Project Period:** 1998/02/01-2006/03/31

Hospital acquired pneumonia costs up to 2 billion dollars per year in the United States, and any inexpensive therapy which reduces this septic complication could greatly impact health care costs. Enteral feeding significantly reduces the complications of pneumonia compared with intravenous (IV-TPN) feedings by 60-70 percent in trauma patients. Our experimental and clinical work implicates previously unrecognized defects in mucosal immunity which develop when the intestinal tract is not stimulated with enteral feeding or when surrogates of enteral feeding are not provided. The principal specific immunologic defense at mucosal surfaces is secretory IgA produced by the mucosal-associated lymphoid tissue (MALT). The principal anatomic site for immunologic sensitization of Peyer's patches within the small intestine. Adhesion molecules direct unsensitized immunocytes through the Peyer's patches where these lymphocytes are sensitized and change their own surface integrins. They are then directed to both intestinal and extraintestinal sites, such as the respiratory tract, where they produce IgA against those antigens. The antibody binds to bacteria, preventing their attachment and their ability to infect. This proposal focuses on how route and type of nutrition affects the expression of the specific adhesion molecules, modified MAdCAM-1, unmodified MAdCAM-1, and ICAM-1 which are important in directing unsensitized immunocytes into Peyer's patches. The proposal tests the hypothesis that interaction between these adhesion molecules and their ligands on naive T and B cells are critical in maintaining mucosal immunity in both intestinal and extraintestinal sites. The proposal is designed to test the hypothesis that inhibition of these interactions recreates the defects in in vivo mucosal defenses that are induced when enteral feeding is not provided. It also focuses on previous observations that a specific immunocyte fuel, glutamine, and the enteric nervous system neuropeptide, bombesin, can act as surrogates for enteral feeding and exert beneficial effects upon the MALT in IV-TPN-fed animals by upregulating MAdCAM-1 and ICAM-1 expression. The experiments are designed to confirm that IgA is a critical element of specific immunity and respiratory defenses against pneumonia with in vivo experiments. These experiments use a monoclonal antibody produced by a hybridoma cell line which is specific for polysaccharide antigen(s) found on a high percentage of clinical isolates of *Pseudomonas aeruginosa*.

**Grant:** 2R01GM053525-06A2  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** SULLENGER, BRUCE A PHD  
**Title:** Analysis of Group I and II Introns in Mammalian Cells  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 1995/09/30-2006/03/31

DESCRIPTION (provided by applicant): The overall goal of this proposal is to explore the ability of group I and group II introns to repair genetic instructions inside mammalian cells. These introns have been of great scientific interest because they are able to perform catalysis and because a subclass of these RNA enzymes can act as mobile genetic elements. Moreover, their ability to modify RNA and DNA sequences through forward and reverse-splicing reactions makes these introns of particular interest to translational researchers. In the previous funding cycle of this grant application, we demonstrated that the *Tetrahymena thermophila* group I intron can perform self- and trans-splicing reactions to alter the sequences of transcripts in mammalian cells. More recently, we demonstrated that the *Lactococcus lactis* group II intron can reverse-splice and insert itself into DNA in transfected human cells. These proof of concept studies suggest that such catalytic RNAs may represent molecules that can be employed to modify genetic instructions for therapeutic ends. These studies also underscore the necessity for further evaluation of these catalytic RNAs in a clinically relevant setting if they are to become therapeutically useful. Herein we propose to perform more detailed analyses of group I and group II intron activity in mammalian cells focusing upon repair of mutant p53 transcripts and genes as a model experimental system. The p53 gene has been chosen as a target for genetic repair in these studies because it is a tumor suppressor gene that is often mutated in human cancers. Moreover, because p53 is a transcription factor, simple and sensitive assays exist to detect p53 activity in mammalian cells. Finally, we have previously demonstrated that a trans-splicing group I ribozyme can repair mutant p53 transcripts and induce p53 activity in a variety of different human cancer cells; thus such an experimental approach will logically build upon our previous work. The completion of these studies will yield a more detailed understanding of group I and group II intron-mediated catalysis in mammalian cells as well as establish the needed experimental foundation from which the logical development of therapeutic group I and group II ribozymes can proceed.



**Grant:** 2R01GM053617-06A1  
**Program Director:** CARTER, ANTHONY D.  
**Principal Investigator:** WU, HAI-YOUNG  
**Title:** GENOME ORGANIZATION: COORDINATED GENE EXPRESSION  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 1996/07/01-2006/03/31

DESCRIPTION (provided by applicant): Our long-term goal is to understand the molecular basis of gene expression control. Toward this goal, we have employed the leucine operon, a bacterial genetic system. Our previous studies have demonstrated that the bacterial *ilvIH-leuO-leuABCD* gene cluster is sequentially activated by a novel promoter relay mechanism. As part of the preliminary studies, we have also demonstrated that the leucine operon is transcriptionally silenced by an AT-rich 318 bp upstream element. Final activation of the leucine operon by the promoter relay mechanism involves derepression (reversal of silencing) through binding of a trans-acting factor, LeuO, to the AT-rich element. Toward understanding the underlying mechanism of silencing and its regulation, we have identified a 47 bp AT-rich (85 percent A+T) DNA, AT8, as the critical silencing component within the 318 bp AT-rich upstream element. The mechanism of AT8-mediated gene silencing in bacteria is remarkably similar to that of eukaryotic gene silencing. First, AT8-mediated gene silencing is position specific and gene non-specific. Second, the bacterial histone-like nucleoid structuring protein (H-NS) appears to be required for AT8-mediated gene silencing, which is reminiscent of heterochromatic gene silencing in eukaryotes. Thus, we hypothesize that a local nucleoid structure modification may be responsible for AT8-mediated gene silencing in bacteria. Using the relatively simple (47 bp) AT8 gene silencer as a model system, we propose to identify all trans- and cis-acting players in gene silencing. In addition, we plan to develop an in vitro gene silencing system for more detailed mechanistic studies. We anticipate that the results of our studies in bacteria will shed light on the mechanism of gene silencing in eukaryotes.

**Grant:** 2R01GM053940-05A2  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** BAUER, CARL E  
**Title:** Genetic Analysis of Mg-tetrapyrrole Biosynthesis  
**Institution:** INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN  
**Project Period:** 1996/06/01-2006/03/31

DESCRIPTION (provided by applicant): The tetrapyrrole biosynthetic pathway is responsible for synthesizing important metabolites such as vitamin B12, hemes, bilins and chlorophylls. The "common trunk" of the pathway, from 5-aminolevulinate to protoporphyrin IX, has received much attention owing to the fact that a number of heredity diseases (porphyrias) are caused by the overproduction of heme precursors. Clinical manifestations of overproducing these intermediates range from simple skin lesions, to psychotic disorders, to death. The vitamin B12 branch of the pathway has also received recent attention genes involved in vitamin B12 synthesis characterized from *Pseudomonas denitrificans* and in *Salmonella typhimurium*. In contrast to the wealth of information on heme and vitamin B12 synthesis, information is just emerging on the synthesis of the Mg-tetrapyrrole family of chlorophylls. In this proposal, we outline plans to perform detailed biochemical and genetic analysis of the Mg-tetrapyrrole biosynthetic pathway. This analysis includes (i) biochemical characterization of enzymes from the Mg-tetrapyrrole branch of the biosynthetic pathway, (ii) biochemical and genetic characterization of a redox responding transcription factor that regulates expression of heme, Mg-tetrapyrroles and carotenoid biosynthesis genes, as well as polypeptides that comprise the light harvesting-II portion of the photosystem. A thorough understanding of the tetrapyrrole biosynthetic pathway has some far ranging practical implications, such as the design of herbicides that target enzymes in the Mg tetrapyrrole pathway, and the health implications of overproducing tetrapyrrole end-products such as vitamin B12 and heme. It should also not be overlooked that tetrapyrrole driven photosynthesis is the primary route of capturing and supplying energy to living cells and, consequently, it is the most important source of energy in our technological world.

**Grant:** 2R01GM053989-06A1  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** DRIKS, ADAM PHD  
**Title:** ASSEMBLY OF THE SPORE COAT OF B SUBTILIS  
**Institution:** LOYOLA UNIVERSITY CHICAGO CHICAGO, IL  
**Project Period:** 1996/05/01-2006/05/31

DESCRIPTION (provided by applicant): Bacterial spores are among the most extraordinary cell types found in nature. These specialized dormant cells are resistant to virtually all forms of environmental assault but retain the capacity to metamorphose into a growing cell as soon as conditions are favorable. These abilities depend on the outermost protective shell that surrounds the spore, a multilayered protein armor called the coat, which gives the spore structural integrity and excludes all large molecules. In spite of the amazing capabilities bestowed on spores by the coat, we know relatively little about how it is built and how it provides protection. In this proposal, we seek to identify the contacts between known coat proteins in *Bacillus subtilis* spores as well as to discover novel coat proteins and the contacts they make within the coat. In particular, we will determine which coat proteins interact with two key proteins, called CotE and SpoIVA, that play important roles in coat assembly. These studies will help us understand the formation of this highly resistant cell type and the basis for its durability. Ultimately, we intend to define the biochemical interactions that direct spore coat assembly. This will provide a broader understanding of the molecular basis of complex assembly events, a question of general relevance to cell biology. The interest of this project is not confined to basic research, however, as bacterial spores from a variety of organisms, particularly clostridia, are major food pathogens and the spores produced by a relative of *B. subtilis*, *B. anthracis*, can be used as a highly effective bioweapon. Much of the potency of these pathogens is due to the coat, which permits rapid spore dispersal and makes decontamination very difficult with current technology. Studies of coat assembly may reveal novel approaches to combat these disease-causing agents.

**Grant:** 2R01GM054090-03A2  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** REUSCH, ROSETTA N  
**Title:** The Role of Polyhydroxybutyrate in OmpA Sorting  
**Institution:** MICHIGAN STATE UNIVERSITY EAST LANSING, MI  
**Project Period:** 1998/05/01-2006/02/28

DESCRIPTION (provided by applicant): Our preliminary studies, which include immuno-, chemical, and enzymatic assays, Edman sequencing, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS), and proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR) indicate that E. coli protein OmpA is modified by cPHB, an amphiphilic, salt-solvating molecule. OmpA is a major outer membrane protein that has served as a paradigm for studies of sorting and assembly of proteins of Gram-negative bacteria into the outer membrane, but despite extensive investigations these mechanisms are still not understood. Previous studies by other investigators have determined that the sorting signal is contained within the eighth B membrane strand. Our preliminary studies indicate that the binding site for cPHB is a tyrosine residue in the eighth f3-strand. Site-directed mutagenesis of this residue produced cPIIB-minus OmpA. OmpA is also widely regarded as a porin, but the question of whether it is capable of forming open pores remains controversial. Liposome-swelling assays and single-channel planar bilayer studies indicate a small percentage of molecules have open pores, whereas high-resolution X-ray structures show no evidence of a continuous water channel through the protein. Our hypothesis is that cPHB-modification is implicated in the sorting and proper membrane assembly and/or pore formation by OmpA. Here we propose to investigate the effects of cPHB on OmpA structure and function. The ability of wild type and cPHB-minus OmpA proteins to incorporate and assembly properly in the outer membrane will be examined in studies of heat-modifiability, sensitivity to proteases, and sensitivity to OmpA-specific phages. The capacity of wild type and cPFIBminus OmpA molecules to form open pores in the membrane will be investigated in single-channel planar bilayer studies. Finally, enzyme(s) responsible for cPHB synthesis in E. coli will be identified.

**Grant:** 2R01GM054098-06A1  
**Program Director:** TOMPKINS, LAURIE  
**Principal Investigator:** BORUKHOV, SERGEI PHD  
**Title:** Structure & Function of Prokaryotic Transcript Cleavage  
**Institution:** SUNY DOWNSTATE MEDICAL CENTER ALBANY, NY  
**Project Period:** 1996/05/01-2005/11/30

DESCRIPTION (Provided by applicant): The prokaryotic transcript cleavage factors GreA and GreB identified in Escherichia coli are thought to have three biologically important and evolutionarily conserved functions in transcription: suppression of elongation arrest, facilitation of promoter escape, and enhancement of transcription fidelity. These functions are accomplished by the ability of Gre factors to induce cleavage of nascent RNA in the ternary transcription complex. The broad goal of this project is to understand the mechanism of action and the structure-function relationships of GreA and GreB. Four types of experiments will be carried out. #1. To identify functionally important localities of Gre factors, we will introduce by oligonucleotide directed random mutagenesis single amino acid substitution of all residues in Gre proteins except those that are involved in the intramolecular interactions. We will also introduce single and multiple amino acid substitutions in the conserved loop and in the region immediately preceding the C-terminal domain by site-directed mutagenesis. The mutants will be characterized by specific transcription assays in vivo and in vitro. #2. To elucidate interactions between Gre and other components of the TC, we will identify mutations in the beta and beta prime subunits of RNAP that suppress the lethal phenotypes of dominant negative mutant Gre factors or overproduction of wt Gre. The mutant RNAPs will be purified, and characterized by in vitro transcription assays. #3. Interactions between Gre proteins and RNA polymerase will be explored using Fe<sup>2+</sup>-induced hydroxyl radical footprinting and mapping, and specific cysteine-directed protein-protein photochemical cross-linking. #4. To obtain three-dimensional structural information, we will prepare crystals of covalently trapped quaternary complex consisting of GreA, DNA template, RNA primer, and the Thermus thermophilus core RNA polymerase and subject them to X-ray crystallographic analysis.

**Grant:** 2R01GM054136-06A1  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** ERIE, DOROTHY A PHD  
**Title:** Kinetic Studies of Transcription Elongation  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 1996/05/01-2006/07/31

DESCRIPTION (provided by applicant): The main goal of the proposed studies is to elucidate the detailed kinetic mechanisms that regulate and control transcription elongation. Transcription, the DNA directed synthesis of RNA, is the first step in the cascade of events that leads to gene expression. The central role of RNA polymerase (RNAP) in transcription is to synthesize the nascent RNA chain with high fidelity and at a reasonable rate. RNAP appears to have evolved such that it catalyzes multiple reactions and displays an unprecedented level of dynamic flexibility. In the past ten years, our understanding of the regulation of transcription at the level of elongation has evolved considerably; however, most of our understanding comes from studies either of static elongation complexes or at positions of regulatory events such as pause and termination sites. As such, the question as to the relevance of these studies to the mechanism of RNA synthesis remains unclear. To understand an enzyme that exhibits such conformational and functional diversity, it is essential to identify all steps in each of the pathways and to determine which step(s) might be rate-limiting and thus subject to regulation. Significantly, only transient-state kinetic methods can identify individual rate-limiting steps. In the previous grant period, using kinetics, we demonstrated that *E. coli* RNAP contains an allosteric binding site in addition to the catalytic site. Binding of the templated nucleoside triphosphate (NTP), but not non-templated NTPs, to this site increases the rate of nucleotide incorporation. The data suggest that RNA polymerase can exist in a state that catalyzes synthesis slowly (unactivated) and one that catalyzes synthesis rapidly (activated), with the transition from the slow to the fast state being induced by binding of the templated NTP to the allosteric site. We hypothesize that this conformational switch is paramount to the regulation of transcription elongation and termination. In the next grant period, we will test many predictions of this model and further investigate the role of NTP binding in regulating transcription elongation. In addition, to develop an integrated model of elongation, we also will characterize transcript cleavage which is important for maintaining accurate and processive synthesis. We will take advantage of the thermal stability and high cleavage activity of *T. thermophilus* RNAP for these latter studies. Finally, the recent publication of crystal structures of yeast RNAP II and *T. aquaticus* RNAP core enzymes bring us into a new era in the study of transcription, providing an unprecedented opportunity to understand the mechanism of RNA synthesis at the atomic level. Accordingly, we will use this information to begin to understand, at the amino acid level, the role of NTP binding and conformational transitions in the regulation of elongation.

**Grant:** 2R01GM055090-05  
**Program Director:** IKEDA, RICHARD A.  
**Principal Investigator:** BECKWITH, JONATHAN R PHD  
**Title:** ANALYSIS OF PROTEIN DISULFIDE BOND FORMATION IN E. COLI  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 1998/02/01-2006/01/31

This proposal is designed to yield new insights into an important feature of protein folding, the process of disulfide bond formation, and to provide biotechnological tools for enhanced production of multidisulfide proteins. I. We will study the process of disulfide bond isomerization in the Escherichia coli periplasm in the following ways: a) We will determine the pathways of electrons from cytoplasm to periplasm that insure the maintenance of disulfide bond isomerases in the reduced state; b) We will determine the specificity of the disulfide bond isomerases, DsbC and DsbG, and of the protein necessary for their reduction, DsbD; c) We will alter the specificity and activity of these proteins using mutagenesis and gene scrambling in order both to understand the mechanism of their action and to enhance the production of heterologous disulfide-bonded proteins; d) We will characterize new genes, which in multi-copy or when altered by mutation, enhance the production of multi-disulfide proteins in the periplasm. II. We will also study the process of disulfide bond formation and isomerization in the cytoplasm of strains of E. coli with altered thiol:disulfide redox environments. Beginning with strains missing the two major thiol:disulfide redox pathways, we have isolated suppressor mutations that restore sufficient reductive power to the cytoplasm for growth, but still allow cytoplasmic formation of protein disulfide bonds. Studies of these suppressors are already revealing novel interactions among the large set of cytoplasmic redox proteins. We will determine the altered genes and the mechanisms whereby suppressor mutations generate the paradoxical reducing but oxidizing cytoplasm. We will analyze suppressor for the kinetics of disulfide bond formation and isomerization and for the efficiency in the production of heterologous disulfide bonded proteins. This proposal thus unites extensive basic studies on processes of electron transfer in thiol: redox reactions with likely benefits for the biotechnological production of medically useful products.

**Grant:** 2R01GM055850-05A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** SHERMAN, DAVID H  
**Title:** Genomic Analysis of Antibiotic Biosynthesis  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 1997/05/01-2006/02/28

DESCRIPTION (provided by applicant): The aim of this competing continuation proposal is to develop a rational approach for metabolic engineering of secondary metabolite production using microbial genomic technologies. In this work both subset and genome-wide microarray methods will be used to analyze secondary metabolism in *Streptomyces coelicolor*. Quantitative physiological and modeling approaches will be combined to obtain information on temporal and conditional expression of global and pathway-specific regulatory factors for antibiotic biosynthetic pathways. In order to dissect further the control architecture in these multi-step biosynthetic systems, key regulatory elements will be investigated and their role in the circuitry of secondary metabolism defined. In addition, controlled expression of structural and regulatory genes in the actinorhodin and undecylprodigiosin biosynthesis will be analyzed to provide a genome-wide understanding of the intricate mechanisms affecting these secondary metabolic pathways. The specific objectives of this project are: I. Perform genome-wide microarray analysis to monitor expression of *absA*, eight *absA*-homologs, and the *cutR/S* and *afsQ1/Q2* two-component regulatory genes involved in secondary metabolite biosynthesis in wild type *S. coelicolor*. II. Construction of the corresponding isogenic mutant strains for each of the two-component regulators noted in Aim I, for subsequent *S. coelicolor* genome microarray analysis. Phenotypic profiling (e.g. growth rate, antibiotic biosynthesis, morphological characteristics) will be performed for each isogenic strain. III. Construction of recombinant *S. coelicolor* strains with engineered regulatory gene::*gfp* fusions to study at the proteomic level temporal and spatial expression patterns for secondary metabolism. From Specific Aims I - III, combine Boolean modeling with data from genomic microarray, mutant phenotype profiling and GFP expression analysis to decode the primary network of regulatory circuits in *S. coelicolor* secondary metabolism. With these methods established, apply high throughput approaches to additional regulatory systems identified using the methods of Aims I -III to establish the detailed layered regulatory network involved in control of antibiotic metabolic pathway gene expression in the *S. coelicolor* genome.



**Grant:** 2R01GM056250-05  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** RODER, HEINRICH PHD  
**Title:** Kinetics of Early Events in Protein Folding  
**Institution:** INSTITUTE FOR CANCER RESEARCH PHILADELPHIA, PA  
**Project Period:** 1998/05/01-2006/04/30

DESCRIPTION (provided by applicant): Many small proteins show evidence for conformational changes prior to the rate-limiting step in the formation of the densely packed native structure. Despite intense study, our understanding of the physical properties and mechanistic role of these early folding intermediates remains incomplete. In particular, little is known about the chain topology and tertiary structural preferences in early intermediates, which are critical for understanding how folding is initiated and directed along productive channels. A major goal of this project is to elucidate the structural properties of the transient states and kinetic barriers encountered during early stages of folding of two representative model proteins, protein G and staphylococcal nuclease. Initial stages of folding extending well into the microsecond time scale will be explored by coupling advanced rapid mixing methods with structurally informative conformational probes, such as intrinsic and extrinsic fluorescence probes, and protection of individual amide hydrogens from solvent exchange monitored by NMR. The involvement of specific residues and interactions in stabilizing transient states and barriers in folding of protein G will be explored by combining these kinetic methods with site-directed mutagenesis. The results will identify key structural features involved in each stage of folding of this prototypic single-domain protein. Detailed insight into the formation of hydrophobic clusters, specific fluorescence quenching interactions and long-range distance distributions during folding of staphylococcal nuclease will be obtained by kinetic analysis of variants with engineered tryptophan residues and fluorescence energy transfer methods. Complementary information on the formation of hydrogen bonded structure during early stages of folding will be obtained by combining ultrarapid quenched-flow methods with NMR-detected hydrogen exchange. The insight into fast folding events for these and other proteins with diverse structural properties will provide a firm experimental basis for testing theoretical and computational models of protein folding, structure prediction and de novo protein design.

**Grant:** 2R01GM056865-05  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** SAYEED, MOHAMMED M PHD  
PHYSIOLOGY:PHYSIOLOGY  
UNSPEC  
**Title:** NEUTROPHIL SIGNALING & EFFECTOR RESPONSES IN BURN INJURY  
**Institution:** LOYOLA UNIVERSITY CHICAGO MAYWOOD, IL  
**Project Period:** 1998/07/01-2006/06/30

DESCRIPTION (provided by applicant):The new knowledge of polymorphonuclear neutrophil (PMN) signaling in relation to effector responses, and their transcriptional and cell-cell interactive capabilities, affords the opportunity to expand investigations of PMN adaptive and inappropriate roles in burn/ trauma and sepsis injury conditions. This study will focus on PMN signaling, cytokine expression and cell-cell interactions during burn injury complicated by *Enterococcus faecalis* infection. The latter organism has been increasingly recognized as a pathogen causing serious morbidity in intensive care patients. Our recent studies have shown that inoculation of *E. faecalis* in burned rats produces hemodynamic instability and mortality not seen with the individual burn or *E. faecalis* challenges. Our previous and preliminary studies have shown burn injury alone causes upregulation of PMNs' signaling, contributing to their enhanced oxidant production, protease release and adhesion to endothelial cells. We hypothesize that PMNs' tissue damaging actions, via oxidant production, protease release, and cell-cell interactions, are exacerbated to produce the animal morbidity and mortality with combined burn and *E. faecalis* injuries. The investigation of mechanisms of PMN's inappropriate potentiated responses will be carried out in a rat model of 30 percent total body surface area burn plus an intraabdominal inoculation of *Enterococcus faecalis* using two strains of the organism, one expressing and one not expressing the AS (Aggregation Substance) protein which has been implicated in enhancing *E. faecalis* virulence. Specific aim 1 will establish linkage between potentiated oxidant production, elastase release, adhesion to endothelial cells, and altered apoptosis of PMNs to the signaling pathways involving protein tyrosine kinases (PTK), phosphatidylinositol 3-kinase (PI-3K), and mitogen-activated protein kinases (MAPK), Erk and p38. The approach in this aim would be to determine if in vivo administrations of signaling blocker agents to the injured animals result in an attenuation/prevention of the potentiated PMN effector response. Specific aim 2 will assess if PMN transmigration across endothelial and epithelial barriers are altered and whether such alterations are related to PMN release of elastase causing damage to the endothelial and/or epithelial adherence junctions by hydrolyzing the junction protein cadherin. Specific aim 3 will evaluate PMN expression of chemokines, CINC, MIP-2 and MIP1  $\alpha$ , and proinflammatory cytokines, IL-1 $\beta$ , IL-6, and their autocrine/paracrine potentiation of PMN chemotaxis and anti-apoptotic behavior. Specific aim 4 is to evaluate the relevance of potentiated effector responses by PMNs in producing damage to structural/functional integrity of intestine, and their relevance to hemodynamic instability and mortality in the burned-*E. faecalis* infected animals. The proposed studies will enhance our understanding of the role of potentiated PMN effector responses in causing host morbidity and mortality in burn injury complicated by enterococcal infections.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 2R01GM057073-05  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** SASISEKHARAN, RAM PHD  
**Title:** Sequence Analysis of Glycosaminoglycans  
**Institution:** MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA  
TECHNOLOGY  
**Project Period:** 1998/02/01-2006/01/31

DESCRIPTION (provided by applicant): Sequencing of DNA and proteins has heralded a biotechnology revolution. Our ability to determine the nucleic acid or polypeptide structure with a specific biological function has enabled us to probe biological phenomenon in a mechanistic, rigorous manner and facilitated the development of novel therapeutics. However, this approach has yet to be achieved with the third major class of biopolymer, viz., polysaccharide. Complex polysaccharides of the glycosaminoglycan (GAG) family are important modulators of numerous biological processes, from development to neovascularization to maintenance of the nervous system. However, except for a few cases, it is still unknown how GAG structures impinge on function. Only with this knowledge will it be possible to utilize the information inherent in GAGs, either scientifically or therapeutically. To this end, the principal investigator has recently developed a powerful sequencing approach for a subset of GAGs (heparan sulfate-like glycosaminoglycans or HLGAGs). One of the primary experimental constraints used in this sequencing approach is the heparinases, a group of polysaccharide lyases from *Flavobacterium heparinum* that the principal investigator has cloned and characterized. In this grant proposal, he proposes to extend the repertoire of tools for use in the sequencing approach and to probe further the biological functions of GAGs. He proposes to do this in two ways: (1) Clone and biochemically characterize other HLGAG-degrading enzymes from *F. heparinum*, and (2) establish a complementary sequencing approach for chondroitin/dermatan sulfate GAGs using the chondroitinases from *F. heparinum*. In this manner, the principal investigator hopes to broaden the knowledge of complex polysaccharides and learn how structure translates to function.

**Grant:** 2R01GM057335-05  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** CRICH, DAVID  
**Title:** Chemical Synthesis of Beta Manno-and Rhamnopyranosides  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 1998/05/01-2006/04/30

Contemporary carbohydrate is being continually challenged by the ever expanding field of glycobiology and, more specifically, by the complexity and diversity of biologically and medically important oligosaccharides uncovered whose synthesis is mandated by their extremely tedious isolation and purification from natural sources in minute quantities. In spite of the remarkable recent advances in the synthesis of carbohydrates, many of which could not have been contemplated more than a few years ago, there still remains many important problems in carbohydrate chemistry to be addressed before the full potential of the field of glycobiology can even begin to be realized. The aims of this project are to provide efficient, effective syntheses of beta-L- and beta-D-rhamnosides and further improved methods for the beta-D-mannosides in both the solution and solid phases. These targets are being pursued with a view to the synthesis of the antigenic capsular polysaccharides from the various strains of *Streptococcus pneumoniae*, all of which have the beta-L-rhamnoside linkage the core of the repeating unit. These capsular polysaccharides are components of multi-valent vaccines used for the prevention of pneumococcal infections, which remain a significant cause of morbidity worldwide especially given the increasing numbers of antibiotic resistant pneumococci. A further aim is to synthesize the common exopolysaccharide repeating units from *Escherichia hermannii* ATCC 33650 and 33650, which has a beta-D-rhamnopyranoside at its core. This is a very different problem, requiring a different solution to that of the *Streptococcus pneumoniae* capsular polysaccharides, as D-rhamnose, unlike L-rhamnose, is not readily available and so cannot be used as starting material. It is intended to develop all of the methods in both the solution and polymer-supported systems. Both have their role to play in the future of carbohydrate chemistry, with the solution methods probably remaining optimal for large quantities of smaller oligosaccharides, and the polymer supported methods being required for the production of pure, longer oligomers. Accordingly, it is necessary that any truly valuable method be demonstrated to be readily transferable between the two phases.

**Grant:** 2R01GM057342-04

**Program Director:** OKITA, RICHARD T

**Principal Investigator:** SIMPSON, LANCE L

PHD

BIOCHEMISTRY:BIOCHEM

RY-UNSPEC

**Title:** Development of Vaccine Carriers

**Institution:** THOMAS JEFFERSON UNIVERSITY

PHILADELPHIA, PA

**Project Period:** 1999/01/01-2006/02/28

DESCRIPTION (provided by applicant): The broad objective of the proposed research is to develop a novel methodology for creating oral and inhalation vaccines. This methodology will utilize the techniques of molecular biology to create fusion proteins that contain two partners: a carrier domain and a vaccine domain. The carrier domain will be a modified and non-toxic variant of botulinum toxin that possesses the ability to cross gut and airway epithelial cells to reach the general circulation. The vaccine domains will be non-toxic polypeptide fragments obtained from tetanus toxin, diphtheria toxin and pertussis toxin. Each of these fragments will have the ability to evoke neutralizing antibodies and therefore resistance against the parent toxins. Initially, the goal of the work will be to create monovalent vaccines (i.e., carrier plus a single immunogen). If this succeeds, a subsequent goal will be to create polyvalent vaccines (i.e., carrier plus a string of two or more immunogens). Generally speaking, experiments will be conducted in a logical progression of steps. The major steps will be: a.) construction of fusion genes that encode carrier and immunogenic components, b.) expression and isolation of the chimeric proteins, c.) evaluation of each putative vaccine to determine whether it crosses gut and/or airway epithelial cells of laboratory animal and human origin, d.) testing of each chimeric vaccine for toxicity when assayed in vitro or in vivo, and e.) testing of each chimeric vaccine to determine whether it will evoke systemic resistance when administered orally or by inhalation to laboratory animals. To be gauged successful, a monovalent or polyvalent vaccine must be able to evoke resistance to at least 1,000 lethal doses of each immunogen in the compound. In the aggregate, the data from this work should indicate whether any particular carrier-immunogen conjugate is worthy of further consideration as a potential human vaccine. If successful, the work should culminate in the generation of monovalent and polyvalent vaccines against tetanus, diphtheria and pertussis toxins. In addition, the work should establish a general approach that could be used to create oral and inhalation vaccines against a broad array of human diseases.

**Grant:** 2R01GM057400-04  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** HARSHEY, RASIKA M PHD  
MICROBIOLOGY:MICROBL  
BIOCHEMISTRY  
**Title:** Signaling during swarmer cell differentiation  
**Institution:** UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX  
**Project Period:** 1998/09/30-2006/02/28

**DESCRIPTION:** (provided by the applicant) The focus of our research is to study signaling mechanisms that lead to differentiation of *S.typhimurium* into hyperflagellated swarmer cells when propagated on a solid growth surface. Knowledge gained from these studies will be extended to the area of biofilms and virulence. A swarmer colony secretes 'slime' which is mainly composed of polysaccharides; biofilms are bacterial colonies within 'slime layers', which play an important role in the persistence of infections. Our current hypothesis is that slime is essential for swarming in at least two ways: provides the milieu for swarming motility, and constitutes the signal for swarmer cell differentiation. Preliminary results have ruled out signals such as specific amino acids, pH changes, oxygen, iron starvation, increased viscosity, flagellar rotation or known autoinducer systems. Extensive transposon mutagenesis has led to the isolation of swarming mutants, a majority of which were defective in lipopolysaccharide (LPS) synthesis, a large number defective in the chemotaxis signaling pathway, and some defective in putative two-component signaling components. A mutation in *waaG* (LPS core modification): secreted copious amounts of slime and showed a precocious swarming phenotype. We have suggested that the O-antigen improves surface 'wettability' required for swarm colony expansion, that the LPS core could play a role in slime generation, and that multiple two-component systems cooperate to promote swarmer cell differentiation. We propose to 1. Investigate the role of the well-understood chemotaxis signaling system in swarmer cell differentiation, 2. Investigate roles of two of the newly implicated two-component signaling systems in swarming and in virulence, and 3. Test polysaccharides as potential swarming signals, and understand the relationship between slime elaborated by moving swarmer cells and that secreted by adherent bacteria in biofilms.

**Grant:** 2R01GM057468-06  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** WANG, PING MD MEDICINE  
**Title:** Sepsis: Mediators and Cardiovascular Responses  
**Institution:** NORTH SHORE-LONG ISLAND JEWISH RES BIRMINGHAM, AL  
INST  
**Project Period:** 1998/05/01-2006/08/31

**DESCRIPTION** (provided by applicant) Despite advances in the management of the septic patient, a large number of such patients die of the ensuing septic shock and multiple organ failure. This mortality might be decreased by a better understanding of the mediators responsible for the transition from the early, hyperdynamic to the late, hypodynamic phase of sepsis so that early detection and modulation of those factors can prevent progressive cell and organ dysfunction. Our studies have indicated that upregulation of adrenomedullin (AM), a recently identified potent vasodilatory peptide, is responsible for initiating the hyperdynamic phase of sepsis. Although vascular AM hyporesponsiveness plays a major role in the transition from the hyperdynamic to hypodynamic phase of sepsis, the reduction of the level of the novel specific AM binding protein (i.e., AMBP-1) and its binding capacity, rather than AM receptors, appear to be the culprit for the vascular hyporesponsiveness. Since co-administration of AM and AMBP-1 prevents the occurrence of hypodynamic sepsis and reduces mortality, we hypothesize that inadequate interaction between AMBP-1 and AM due to the reduction of AMBP-1 plays an important role in the development of the hypodynamic response, multiple organ failure, and mortality during the progression of sepsis. Thus we propose two specific aims. Aim 1: To determine mechanisms responsible for producing the vascular AM hyporesponsiveness in sepsis. We will examine how sepsis affects AMBP-1 biosynthesis, its binding capacity, and AM bioactivity. The role of proinflammatory cytokines (TNF- $\alpha$ , IL-1B) and endotoxin will be examined. Studies are proposed to determine whether Kupffer cell activation suppresses AMBP-1 synthesis and release. Aim II: To determine mechanisms by which administration of AM/AMBP-1 maintains cardiovascular stability in sepsis. We will examine whether AM/AMBP-1 affects Amreceptor binding capacity, signal transduction pathways, and endothelial constitutive nitric oxide synthase (ecNOS). The role of inhibition of vascular endothelial cell apoptosis and shedding as well as TNF-A and IL-B production in producing AM/AMBP-1's beneficial effects will be studied. Moreover, the effects of delayed infusion of AM/AMBP-1 on organ function and survival will be assessed. The proposed studies should provide useful information which will allow us not only to understand the mechanisms responsible for transition from the hyperdynamic to hypodynamic sepsis, but also for maintaining cardiovascular stability and preventing organ failure and mortality.



**Grant:** 2R01GM057720-31

**Program Director:** ANDERSON, JAMES J.

**Principal Investigator:** DUBNAU, DAVID A PHD  
GENETICS:BIOCHEMICAL/M  
LECULAR

**Title:** Regulation of genetic competence in *Bacillus subtilis*

**Institution:** PUBLIC HEALTH RESEARCH INSTITUTE NEW YORK, NY

**Project Period:** 1977/06/01-2005/11/30

DESCRIPTION (Provided by applicant): Genetic competence is defined as a state in which bacterial cells are able to bind and internalize high molecular weight DNA, which may then be established as an independent replicon or recombine with a resident DNA molecule. Competence in *Bacillus subtilis* is one of several post-exponentially expressed systems, the most extensively studied of which is sporulation. The present study is directed towards unraveling the complex regulatory network that determines the onset of competence expression in response to environmental signals. This network is known to rely on quorum sensing, transmembrane signaling, phosphorylation events involving two-component regulators, protein-protein and protein-DNA interactions, transcriptional and posttranscriptional regulation as well as the action of ClpC, an HSP100-like protein. We will investigate the response of ComP to quorum-sensing ComX pheromones and we will determine the structure of the pheromone modification. We will study the interactions of MecA, ClpC and ComS which regulate the stability of the competence-specific transcription factor ComK. We will investigate the interactions of the five proteins known to bind at the comK promoter. We will study the factors responsible for the differentiation of two cell types in a competent culture, and the competence-specific growth arrest. Finally we will use expression profiling with genomic microarrays to study the global effects of mutations in competence regulatory genes. Quorum sensing and the ClpC heat shock protein regulate pathogenesis in a number of bacterial pathogens. Understanding the biology of the proteins involved may help to clarify the virulence mechanisms involved.

**Grant:** 2R01GM057755-20  
**Program Director:** TOMPKINS, LAURIE  
**Principal Investigator:** GROSS, CAROL A  
**Title:** MUTATIONAL ANALYSIS OF E COLI CORE RNA POLYMERASE  
**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO  
SAN FRANCISCO, CA  
**Project Period:** 1983/01/01-2005/12/31

DESCRIPTION (Provided by applicant): Our fundamental interest is the central biological problem of how the cell regulates transfer of information from gene to protein. RNA polymerase not only carries out transcription, but also integrates intracellular and extracellular signals by adjusting its transcriptional potential to the sum of the regulatory inputs. To understand how various cellular regulatory systems control RNA polymerase, our approach has been to first delineate the functional anatomy of the enzyme and then investigate how altering its individual functions affects gene regulation. We continue this approach with a focus on how the interaction between RNA polymerase and its initiation factors conveys information to each partner. In addition, we initiate a new approach: genome-wide analysis. Following perturbations that alter the interaction of initiation factors and regulators with RNA polymerase, we will be able, for the first time, to correlate functional changes in RNA polymerase with global changes in gene expression. During the current granting period we will: 1. Identify the roles of sigma 70-core contact sites in RNA polymerase function 2. Use minimal assemblies to identify the functions of contacts between sigma 70 and core. 3. Test the sequential interaction model for the interface between sigma and core. 4. Further define the interface between sigma 70 and core. 5. Determine global effects of transcriptional apparatus alterations using microarray analysis. These studies provide a paradigm for how initiation factors and RNA polymerase prepare each other for initiation in all prokaryotes, including pathogens. Moreover, given the extensive structural conservation between prokaryotic and eukaryotic RNA polymerase, especially near the active site, our findings are likely to have general applicability to all organisms. Finally, our studies on the circuitry governing expression in *Escherichia coli* will be a useful blueprint as attention switches to newly determined genomic sequences of pathogens lacking the rich history of genetic and biochemical investigation that is enjoyed by *E. coli*.

**Grant:** 2R01GM057846-05  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** PEROZO, EDUARDO PHD  
**Title:** High Resolution Structural Dynamics of K Channels  
**Institution:** UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA  
CHARLOTTESVILLE  
**Project Period:** 1998/08/01-2006/08/31

DESCRIPTION (provided by applicant): In response to membrane potential depolarization, voltage-dependent potassium channels undergo a series of conformational changes from a non-conducting state (closed) to an activated (conducting) state. K<sup>+</sup> channel function has been associated with such basic cellular functions as the regulation of electrical activity, signal transduction and osmotic balance. In higher organisms, K<sup>+</sup> channel dysfunction may lead to uncontrolled periods of electrical hyperexcitability, like epileptic episodes, myotonia and cardiac arrhythmia. Consequently, efforts to understand K<sup>+</sup> channel structure, function and dynamics relate directly to human health and disease. The continuing long-term goal of this project is to further understand the molecular mechanisms of gating in voltage-dependent channels, by focusing on the analysis of K<sup>+</sup> channel gating. This understanding encompasses two interrelated processes, the protein rearrangements that lead to channel opening and the energy transduction events that convert external stimuli (voltage, ligand binding, etc) into protein motion. Specifically we will address the following key questions: What are the molecular entities determining channel activity? How energy (in the form of specific ligand binding or transmembrane electric field) is transduced into protein motion? How different parts of the channel interact to define open channel activity? We plan to study these problems by combining site-directed spin labeling/EPR spectroscopy and electrophysiological methods with classical biochemical and molecular biological procedures. This particular strategy has proven very successful over the previous application period, leading to direct structural determinations of KcsA, the *Streptomyces* K<sup>+</sup> channel, the types of molecular movements underlying its gating mechanism and structural information on the role of the selectivity filter in gating. We intend to continue these structure-function studies while extending them using new experimental approaches like Double Quantum Resonance FT-EPR. In addition, we will focus our attention on a newly characterized six-transmembrane segment (6TM) channel from *Methanococcus janschii* (which we have named KchV-O). This channel contains a bona fide S4 segment and is ideally suited to study the structure and dynamics of the voltage-sensing domain and voltage-dependent gating mechanisms. This proposal should open new experimental avenues that will contribute to our understanding of biologically important events such as electrical signaling, signal transduction and ion channel gating.

**Grant:** 2R01GM057917-05  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** MILLER, JEFFREY H PHD  
**Title:** Genomics of High Temperature Biological Systems  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 1998/08/01-2006/07/31

DESCRIPTION (provided by applicant): Numerous members of the third kingdom, Archaea, are extreme hyperthermophiles. Such organisms pose many interesting fundamental questions in molecular biology, since all of their life processes occur at elevated temperatures. For instance, how do regulatory proteins recognize and bind to operators at temperatures above 100 degrees C? How can the DNA replicate with high fidelity, and how do these organisms avoid excessive mutation due to heat-induced DNA lesions, and what repair strategies do they employ? We propose to develop a functional genomic analysis of one of the high temperature archaea, *Pyrobaculum aerophilum*, that can grow up to 104 degrees C, with an optimal growth temperature of 100 degrees C. We have sequenced and fully annotated the 2.2 mega base genome of *Pyrobaculum aerophilum*, and are completing the construction of microarrays containing every ORF, in collaboration with Todd Lowe at Stanford/U.C. Santa Cruz. We will carry out experiments on genome-wide gene expression in different environments, and use the data to identify coregulated genes. Through extended analyses, we hope to use the data to set up experiments to identify regulatory pathways and regulatory proteins. We will extend the development of a genetic system with the aim of being able to carry out reverse genetics in this organism. We have begun an indepth investigation of the repair systems in this organism, and we will continue to characterize DNA repair strategies and proteins.

**Grant:** 2R01GM058133-05  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** LEIGHTON, JAMES L PHD  
**Title:** Asymmetric Synthesis of Macrolide Antibiotics  
**Institution:** COLUMBIA UNIV NEW YORK MORNINGSIDE NEW YORK, NY  
**Project Period:** 1998/08/01-2006/07/31

DESCRIPTION (provided by applicant): This proposal details the development of new, catalytic reaction methodology for the efficient asymmetric synthesis of polyacetate- and polypropionate-derived macrolide antibiotics. It has been documented that drug-resistant bacterial and fungal infections represent a problem of rapidly growing importance. More than simply achieving syntheses of such compounds, the focus of the proposal is on advancing the frontiers of efficiency, waste-minimization and economic viability of such synthetic efforts. The ultimate goal is thus the realization of practicable and practical chemistry that will affect both the discovery and process phases of research into new medicinal agents. This proposal addresses both the continuation and further development of the most promising discoveries made in the previous funding period, as well as exciting new directions only recently initiated. The reactions under study have as their unifying theme tandem reaction methodology. Tandem reactions are inherently more efficient as multiple chemical transformations may be accomplished, and multiple stereogenic centers may be established in a single operation. In this fashion, large segments of the target natural products may be synthesized with unprecedented efficiency employing only readily available and environmentally benign reagents.

**Grant:** 2R01GM058213-05  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** KOLTER, ROBERTO G PHD BIOLOGY  
**Title:** Molecular Genetics of Biofilm Formation  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 1998/09/01-2006/08/31

DESCRIPTION (provided by applicant): Populations of surface-attached microorganisms are commonly referred to as biofilms. In most natural settings bacteria are found predominantly in biofilms, yet for many years studies of bacterial physiology focused on the planktonic state of bacterial cells. The widespread recognition that biofilms impact a myriad of environments has led to concerted efforts to gain a better understanding of the molecular mechanisms that underlie the development of these communities. Recent results have revealed that biofilm formation is a complex developmental process that occurs in response to environmental cues. Working models for how planktonic bacteria proceed from environmental sensing to the formation of mature biofilms are now guiding many investigators in their research. However, most of the attention has been placed on biofilms that form on abiotic solid surfaces such as plastics and glass. The formation of biofilms on living surfaces is also widespread and has important impacts in environmental and clinical settings. In our first experimental approach we propose to extend the studies we have carried out with a model bacterium, *Pseudomonas aeruginosa*, to investigate how it forms a biofilm on living fungal filaments. To this end we will: 1) Characterize the bacterial-fungal interactions through physiological, biochemical and microscopic analyses, 2) Select specific genes in which to generate mutations and test their effects on bacterial-fungal interactions and 3) Carry out a mutant screen and perform transcriptional profiling using microarrays to identify additional genes involved in the bacterial-fungal interactions. It is generally accepted that there is cellular differentiation within biofilms. Yet, relatively little is known about the molecular mechanisms that underlie differentiation processes in biofilms. In a second experimental approach that follows a path analogous to our first approach, we will address the question of cellular differentiation in biofilms through the study of a well-characterized sporulation process in *Bacillus subtilis*. We will: 1) Analyze the spatial and temporal patterns of transcription of a spore-specific gene using reporter fusions and light, electron and confocal scanning laser microscopic techniques, 2) Generate additional reporter gene fusions to selected genes and analyze their spatial and temporal patterns of expression in order to develop a more complete functional anatomy of the biofilm and 3) Test the effects of mutations involved in cell-cell signaling and environmental sensing on cellular differentiation within the biofilm.

**Grant:** 1R01GM061017-01A2  
**Program Director:** WOLFE, PAUL B.  
**Principal Investigator:** SCHILDBACH, JOEL F PHD  
**Title:** MOLECULAR ANALYSIS OF TRAI (E. COLI HELICASE I) FUNCTION  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/04/01-2007/03/31

**DESCRIPTION:** (provided by applicant): Conjugation is an important means of genetic transfer between bacterial species and has been implicated in the spread of genes encoding antibiotic resistance. The goal of this research program is to understand the mechanism and regulation of plasmid nicking and of initiation of DNA transfer in bacterial conjugation. Plasmid nicking, a prerequisite of transfer of a single plasmid DNA strand, is performed by relaxases, nucleases that bind and cleave single-stranded DNA. TraI, the relaxase of conjugative plasmid F Factor, is essential to F conjugative transfer. TraI possesses both a relaxase activity and a helicase activity, the latter unwinding and separating the plasmid DNA strands as the cut strand is transferred to the recipient. How TraI is able to act against double-stranded DNA in vivo, and how TraI converts between its relaxase and helicase activities are unknown. Experiments are proposed to answer four key questions about TraI and conjugation initiation: How is TraI nicking and transfer initiation influenced by oriT DNA sequences and the proteins that bind them? What protein-protein interactions influence TraI nicking and the initiation of DNA transfer, and what is the effect of disrupting them? How does a protein recognize single-stranded DNA with exquisite sequence specificity? How does TraI structure and domain organization influence its activity? These questions will be answered through in vivo studies that will identify TraI proteins, protein interactions and plasmid DNA sequences that influence transfer, and will determine the effects of these proteins and DNA sequences on plasmid nicking, transfer initiation, and transfer termination. In vitro studies will focus on determining the DNA sequences and distortions that TraI can recognize, the energetics of the TraI-DNA interaction, the conformational or functional events that occur subsequent to TraI binding to DNA, and the role of structure and domain organization in determining TraI function.

**Grant:** 1R01GM061020-01A2  
**Program Director:** WOLFE, PAUL B.  
**Principal Investigator:** MATSON, STEVEN W PHD  
BIOCHEMISTRY:NUCLEIC  
ACID  
**Title:** Mechanism of Conjugative DNA Transfer  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2002/04/01-2006/03/31

DESCRIPTION (Provided by applicant): Conjugative transfer of genetic traits is mediated by a wide range of plasmids and transposons, and can occur between species and even kingdoms. Although first described over 50 years ago, we still have only a rudimentary knowledge of the molecular details surrounding this important mechanism for DNA transfer. Detailed knowledge of the conjugative mechanism is, therefore, of critical importance. The long range goal of this project is to understand, at a molecular level, the mechanistic details of conjugative DNA transfer. Previous studies indicate that DNA transfer begins at a site- and strand-specific nick (nic) in the conjugative plasmid, which is then unwound as ssDNA is transferred into the recipient. This laboratory has shown, using the F plasmid as a model, a requirement for two F-encoded proteins, Traip and TraYp, and one host-encoded protein, integration host factor (IHF), in the nicking reaction; subsequent unwinding has not yet been reconstituted in any system. Four specific aims are proposed. The 1st aim will focus on the role of TraYp and IHF in the TraIp-catalyzed transesterification reaction. Preliminary data suggest IHF may bind to one of two mutually exclusive sites that provide a molecular switch for initiating conjugation that is either on or off. This will be explored using chemical footprinting and IHF binding site mutants. In addition, TraYp + tHE may alter the DNA structure surrounding nic such that the DNA has ssDNA (or non-B DNA) character. The 2nd aim is to reconstitute the coupled nicking-unwinding reaction catalyzed by TraIp. Initial studies indicate a previously unrecognized host protein is required to "trigger" unwinding of DNA nicked by TraIp. This protein will be purified, using a biochemical complementation assay, and characterized in terms of its interaction with TraIp and its role in strand transfer. The F plasmid model provides the best possibility of reconstituting this key reaction because the helicase and site-specific nicking activities have been identified and a minimal relaxosome has been reconstituted. The 3rd aim will define the catalytic residue(s) in Traip involved in the site- and strand-specific transesterification reaction. Preliminary results indicate the involvement of two tyrosines, Y16 and Y23. The role of each tyrosine will be evaluated by constructing specific mutants and evaluating each mutant in vitro and in vivo. We also propose to crystallize the TraIp transesterase domain in the presence and absence of an oligonucleotide substrate to gain insight into the interaction of the transesterase with its substrate. The final aim will focus on the role of TraMp in the initiation reaction. Genetic studies indicate a role for this protein but biochemical details are lacking. The protein will be purified and used in relaxosome reconstitution studies. Taken together, the results gained from these experiments will advance our understanding of the mechanism of conjugative DNA transfer and will pave the way



for future experiments to look at transfer across the cell membrane.

**Grant:** 1R01GM061656-01A2  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** WARD, PETER A MD INTERNAL  
MED:INTERNAL MEDICINE  
UNSPEC  
**Title:** Protective Effects of Anti-C5a in Sepsis  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/08/15-2006/05/31

DESCRIPTION (provided by applicant): On the basis of our studies to date using the experimental model of sepsis induced by cecal ligation/puncture (CLP) in rats, serious impairment of innate immunity develops. This results in what appears to be a C5a-dependent defect in assembly of NADPH oxidase and defective phagocytic function of neutrophils. These defects can be reproduced by in vitro exposure of neutrophils to concentrations of C5a found in sepsis. In the first aim, we will evaluate how in vitro exposure of neutrophils to C5a results in defective signaling pathways: phorbol 12-myristate 13-acetate (PMA)-induced activation of phosphokinase C (PKC) which results in assembly of NADPH oxidase; and cell activation by engagement of FcyRs resulting in phagocytic responses. In the second aim, we will evaluate the same signaling pathways in blood neutrophils from CLP animals and determine if treatment with anti-C5a prevents defective signaling. In the third aim, we will determine if treatment of normal rats and mice and CLP rats and mice with anti-C5a compromises innate immunity, as assessed by bacterial clearance (*Pseudomonas* sp. and *Klebsiella* sp.) from lungs and evaluate the effects on survival. In the fourth aim, we will employ microarray analysis in CLP rats to define, as a function of time, alterations in global gene expression in organs that are predisposed to injury during sepsis (liver, lungs, kidneys, thymus) and determine if treatment with anti-C5a prevents this pattern of gene expression. It is possible that microarray analysis will be predictive of organ susceptibility to damage during sepsis. Collectively, these studies should provide important evidence related to the mechanisms by which complement activation during sepsis impairs innate immunity.

**Grant:** 1R01GM061748-01A2  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** MAGUIRE, MICHAEL E  
**Title:** Manganese Homeostasis and Salmonella  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 2002/01/11-2005/12/31

NRAMP proteins (Natural Resistance Associated MacroPhage Protein) are H<sup>+</sup>-stimulated divalent cation transporters. Mammalian NRAMP2/DCT1 mediates intestinal uptake of Fe<sup>2+</sup> and Mn<sup>2+</sup> and distribution of these cations within cellular compartments. NRAMP1 is expressed in macrophages and is the long known Ity/Lsh/Bcg locus in the mouse that provides resistance to diverse pathogens such as *S. typhimurium*, *Leishmania donovani* and *Mycobacterial* species. Polymorphisms in human NRAMP1 render the bearer more susceptible to *M. tuberculosis* infection. NRAMP proteins have been characterized primarily in eukaryotic cells; however, many Gram<sup>+</sup> and Gram<sup>-</sup> bacterial species have close homologs (40% sequence identity), suggesting conservation of function. *E. coli* and *S. typhimurium* carry a single NRAMP gene which we have cloned and characterized. Our data indicate that a) the bacterial NRAMP protein is a highly selective H<sup>+</sup>-stimulated Mn<sup>2+</sup> transport system (mntH) whose expression is regulated by peroxide and divalent cation. b) MntH is highly induced upon *S. typhimurium* invasion of NRAMP1<sup>+</sup> but not NRAMP1<sup>-</sup> macrophages, c) virulence of mntH is attenuated in NRAMP1<sup>+</sup> mice but not NRAMP1<sup>-</sup> mice, d) the putative iron transporter SitABCD is a physiologically relevant Mn<sup>2+</sup> transporter and 3) mutation of both mntH and sitABCD renders *S. typhimurium* avirulent. If abrogation of Mn<sup>2+</sup> uptake results in loss of virulence, then Mn<sup>2+</sup> itself is needed at some point in pathogenesis. In Aim 1, we will ask where, when and why Mn<sup>2+</sup> is important for pathogenesis. Where and when will be determined by investigation of the effect of mutations in mntH (and sitABCD) on invasion, survival and proliferation of *S. typhimurium* in NRAMP1<sup>+</sup> and NRAMP1<sup>-</sup> macrophages and also epithelial cell lines. Virulence will be determined in congenic NRAMP1<sup>+</sup> and NRAMP1<sup>-</sup> BALB/c mice. Why will be investigated by examining a subset of the very few Mn<sup>2+</sup>-dependent enzymes: superoxide dismutase (sodA), protein phosphatases 1 and 2 (prpA/B), and phosphoglyceromutase (gpmM). The effects of mutations at these loci will be measured on invasion of cultured cells and virulence in mice. We will also investigate regulation of mntH expression by Fur, OxyR and a new DtxR homolog MntR. In Aim 2, we will determine the topology of MntH, further examine its transport properties using <sup>54</sup>Mn<sup>2+</sup> as tracer and also electrophysiologically after expression in *Xenopus* oocytes, and investigate the role of conserved and charged intramembrane residues in cation flux by site-directed mutagenesis.

**Grant:** 1R01GM062351-01A1  
**Program Director:** ECKSTRAND, IRENE A.  
**Principal Investigator:** DESALLE, ROB PHD  
**Title:** Genetic Transfer in the Evolution of Infectious Disease  
**Institution:** AMERICAN MUSEUM OF NATURAL HISTORY NEW YORK, NY  
**Project Period:** 2002/02/01-2006/01/31

**DESCRIPTION** (provided by applicant): It is widely acknowledged that the acquisition of foreign DNA elements by Bacteria has contributed significantly to the emergence of new pathogens. Indeed, horizontal transfer of is believed to be an important process in the evolution of many microorganisms. Transferred genes can provide traits that confer a selective advantage to pathogens and confer virulence on non-pathogens. Genes for antibiotic resistance, toxin production, and host-specificity can all be acquired horizontally. Though much is understood about the mechanical processes of horizontal transfer in bacterial model systems, less is known about the historical dynamics of horizontal transfer and its impact on the phenotype and genomes of host organisms. Phylogenetic analysis offers a powerful framework to identify horizontal transfer and test hypotheses about its evolutionary dynamics and implications. We propose to adapt existing phylogenetic techniques and develop new ones to investigate at high resolution the process of horizontal transfer and its impact on the evolution of infectious disease. We also propose to use molecular genetic methods in the laboratory to test phylogeny-based predictions about horizontal transfer. To analyze gene transfer, it is critical to examine a system that can be horizontally acquired, is widely distributed, and allows for dense taxon sampling. The highly conserved Widespread Colonization Island (WCI) presents an unparalleled model system to test hypotheses and develop methodologies to better understand horizontal transfer. The WCI, first identified in the periodontal pathogen *Actinobacillus actinomycetemcomitans*, contains genes essential for the tenacious, nonspecific adherence of the bacterium to surfaces, and fibril biogenesis. Sequence analysis shows that the WCI is distributed in a wide array of pathogens and nonpathogens that inhabit very distinct niches. Preliminary phylogenetic analyses and other evidence suggest that the WCI has experienced multiple horizontal transfer events. The WCI will be used as a model system to study the process of horizontal transfer. Our objectives are: (1) to test and develop methods that can reliably identify historical transfer events, the donor and recipient lineages involved, and the relative dates of their occurrence; (2) to develop phylogenetic techniques and methods for genome analysis to understand the emergence of pathogenicity due to horizontal transfer; (3) to understand the co-evolution of the components of a genetic island with each other and with their bacterial host(s); (4) to identify genetic or phylogenetic barriers to the transfer of pathogenicity islands among hosts and; (5) to design accessible, computer-based phylogenetic and genomic methods for the analysis of horizontal transfer and host/parasite co-evolution. The WCI system offers an outstanding opportunity to understand horizontal transfer on a large scale and to develop new methodologies that can be generalized for the analysis of horizontal transfer, infectious disease, and the evolution of virulence in other systems.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R01GM062449-01A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** BARABASI, ALBERT-LASZLO PHD  
**Title:** Topologic properties of metabolic networks  
**Institution:** UNIVERSITY OF NOTRE DAME NOTRE DAME, IN  
**Project Period:** 2002/02/01-2005/01/31

Over the past several decades, the biomedical research community has acquired an enormous amount of valuable data across a wide spectrum of the biological world. Nearly all these efforts reflect reductive, analytical approaches to investigating important biological questions, in which biologists typically have deliberately restricted their analyses to well-defined systems with relatively few components, implicitly attempting to reduce biological phenomena to the behavior of individual molecules. Yet, despite the value of these approaches, a discrete biological function cannot be attributed only to individual molecules. Instead, the robust behavior of biological systems arises to a large extent from complex interactions among its various building constituents (i.e., cellular networks). In this application, we propose to conduct a highly integrated program in which we will examine in quantitative terms the structure of complex metabolic networks that are required to maintain the proper function of a cell. This goal will be aided by two recent scientific developments: the emergence of integrated pathway-genome databases providing detailed connectivity maps of metabolic networks, and by theoretical advances in comprehending and quantifying the topology of complex (non-biological) networks. This research represents a unique collaboration between a theoretical physicist (A. -L. Barabasi), a physician-molecular biologist (Z. N. Oltvai), and a bacterial molecular geneticist (B. L. Wanner). Our aims are threefold: (1) We will analyze in quantitative terms the structure and functional activity of complex metabolic and genetic networks of model organisms, such as *Escherichia coli*; (2) We will examine the effects of perturbing the levels of proteins in central metabolic networks to aid in model building and to test rules that evolve from computer simulations of these models, including examining their tolerance to targeted mutations; and (3) We will attempt to develop an understanding of the dynamic changes that take place in metabolic networks in response to a changing environment, in studies of the *Escherichia coli* physiome. Understanding the principles of interactions among various metabolic network components of a living cell and the generic large-scale feature of metabolic networks will not only provide an important contribution to basic biology, but will also have applicability to translational research, such as pharmaceutical target identification.

**Grant:** 1R01GM062630-01A1  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** PERONA, JOHN J PHD  
**Title:** Transcriptional Control of the E. coli Pap Operon  
**Institution:** UNIVERSITY OF CALIFORNIA SANTA BARBARA, CA  
BARBARA  
**Project Period:** 2002/01/01-2005/12/31

DESCRIPTION (provided by applicant): A multidisciplinary study aimed at uncovering the structural and energetic basis for transcriptional activation in the E. coli pap operon is proposed. Genetic, biochemical and X-ray crystallographic experiments will be employed to test an elegant model for regulation known as the phase variation control mechanism. This model invokes the DNA methylation state of two upstream GATC sequences as a key factor determining which of six possible binding sites are occupied by the leucine-responsive regulatory protein (Lrp). The translocation of Lrp dimers along the DNA, in a process mediated by the coactivator protein PapI, is essential for RNA polymerase binding and subsequent transcription. Rigorous biochemical experiments will provide a comprehensive set of binding free-energy parameters describing the inherent DNA sequence preference of the Lrp protein for individual sites, together with how this is modulated by the physiologically relevant effectors. Several in vivo genetic selections will also be applied to Lrp, to identify specific amino acids involved in the responsiveness to DNA methylation and to PapI binding. Finally, X-ray crystallography will be used to determine the high-resolution atomic structure of unliganded Lrp, of the binary Lrp-DNA complexes with several different single-sites, and of the ternary Lrp-PapI-DNA complex. The analyses promise considerable general insight into the basis of methylation effects on protein-DNA interactions, as well as the interplay of protein-induced and intrinsic A-tract bending in the formation of higher-order nucleoprotein structure. The pili proteins regulated by this operon are essential for targeting of bacteria to the surface of uroepithelial cells lining the human urinary tract. Detailed information on pap regulation should thus be helpful in designing strategies to inhibit host colonization and pathogenicity.

**Grant:** 1R01GM062842-01A1  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** PORCO, JOHN A BA  
**Title:** Synthesis of Enamide and Related Natural Products  
**Institution:** BOSTON UNIVERSITY CHARLES RIVER BOSTON, MA  
CAMPUS  
**Project Period:** 2002/01/10-2006/12/31

**DESCRIPTION:** (provided by applicant) This research proposal reflects our interest in the areas of new reaction methodology development, applications to the synthesis of biologically active natural products, and their mechanism of action. We intend to develop and further refine synthetic methodologies for the synthesis of bioactive enamides, as well as related N-acylheminal natural products. We plan to study the chemical reactivity and biological activity of these molecules via specific biological collaborations. In addition to methodology development, the synthesis of structurally complex and biologically active natural products will be pursued: We intend to complete the synthesis of the salicylate macrolide lobatamide C in order to assign the absolute stereochemistry at unknown stereogenic centers, evaluate the biological activity of synthetic intermediates, and prepare both stereochemical analogues and photoaffinity probes. We intend to synthesize the cytotoxic peptide enamide natural products chondriamide C and terpeptin and diverse analogues using chemical modification of C-terminal peptides. We intend to pursue the asymmetric synthesis of novel antitumor and cell-cycle inhibitory macrolide oximidine I. We will pursue the asymmetric synthesis of the antitumor compound zampanolide whose natural resource has been depleted.



**Grant:** 1R01GM062899-01A2  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** TALAMINI, MARK A MD  
**Title:** REDUCED INFLAMMATORY RESPONSE DURING LAPAROSCOPY  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/07/01-2006/06/30

DESCRIPTION (provided by applicant): Laparoscopic surgery represents a new paradigm, with less pain, quicker recovery times, and reduced disability. In time, most general surgical procedures will be performed in this manner. Laparoscopic surgery is not simply a reduced surgical insult, it is a different surgical insult. Preliminary data in the rat shows that a pneumoperitoneum with CO<sub>2</sub> gas alters the inflammatory response associated with laparoscopic surgery in the setting of sepsis. The objective of this application is to establish whether CO<sub>2</sub> pneumoperitoneum has a modifying role, and to determine the possible mechanism(s). AIM 1: To test whether CO<sub>2</sub> modifies the inflammatory response during laparoscopic surgery. The effect of pneumoperitoneum with CO<sub>2</sub>, helium, or air on the inflammatory response after sepsis (cecal ligation and puncture) will be compared. The parameters of the inflammatory response to be evaluated are: leukocytosis, leukocyte infiltration, hepatic acute phase gene expression, and circulating levels of cytokines. AIM 2: To determine where in the pathway of the inflammatory response CO<sub>2</sub> pneumoperitoneum exerts its effect. Expression of the acute phase proteins will be used as an experimental paradigm. Pre-transcriptional, transcriptional, and post transcriptional regulation of the acute phase genes will be evaluated. AIM 3: To determine how the CO<sub>2</sub> pneumoperitoneum alters the inflammatory response. The hypothesis of this aim is that alteration of pH with abdominal distension alters the inflammatory response. A) Arterial pH will be measured to correlate pH changes with immune modulation. B) The pH will be artificially decreased by a comparable amount via systemic intravenous infusion of hydrochloric acid in the setting of sepsis plus CO<sub>2</sub> pneumoperitoneum, helium pneumoperitoneum, air pneumoperitoneum, and open surgery. The information provided by this investigation will be essential in patient care as increasing numbers of patients undergo operations using the CO<sub>2</sub> pneumoperitoneum.

**Grant:** 1R01GM062954-01A1  
**Program Director:** IKEDA, RICHARD A.  
**Principal Investigator:** DIKANOV, SERGEI PHD  
**Title:** Structure-function relations in Rieske-type proteins  
**Institution:** UNIVERSITY OF ILLINOIS URBANA- CAMPAIGN, IL  
CHAMPAIGN  
**Project Period:** 2002/03/01-2007/02/28

Iron-sulfur proteins are ubiquitous in nature and occur in most metabolic pathways, and an understanding of the parameters determining their physico-chemical properties is a central problem of biochemistry. Among the most interesting are the 2Fe-2S clusters that have as a common feature the liganding of the irons by two cysteines and two histidines. These include the Rieske iron-sulfur protein (ISP) of the bc1 (and related) complexes, and several Rieske-type bacterial proteins, including the archaeal sulredoxin (SDX) of *Sulfolobus* sp. Strain 7 and Rieske-type ferredoxin (ARF) from *S. solfaricus* to be investigated in this project. A remarkable feature of these proteins is the wide variation in redox potentials, from -100 to 300 mV, exhibited by the 2Fe-2S clusters, despite their similar ligation. Recent crystallographic structures show a similar orientation of ligands in the cluster binding domain in proteins at both extremes of this range. This implies that the redox potentials and protolytic properties of each particular cluster are controlled by other unique features of the protein environment. To understand how the proteins function, the factors influencing these parameters must be determined at the atomic level for each protein. We propose to investigate the protein environment of the three Rieske-type clusters above by using advanced magnetic resonance techniques, with an emphasis on 2-D ESEEM to explore the interaction between the paramagnetic centers and nuclear spins in the neighborhood. We will analyze the data so as to provide structural information, and compared this with the crystallographic structured of ISP, and of naphthalene- 1,2-dioxygenase. The spectroscopic results will provide constraints to allow precise determination of the cluster environment including coordination of histidine and cysteine ligands, presence of hydrogen bonds and non-coordinated nitrogens, and accessibility of solvent. By performing similar experiments with mutant strains generated by molecular engineering, we anticipate that we will be able to identify the local features of the protein environment that control the redox and protolytic properties of the clusters, their role in reaction mechanisms, and the changes that produce functional modification in different strains. The results will answer some fundamental questions about structural factors controlling the redox potentials of Rieske-type proteins, and provide insights to similar questions in other redox proteins.

**Grant:** 1R01GM063075-01A2  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** WANG, HAICHAO PHD  
**Title:** Regulation of HMG-1 Release in Endotoxemia  
**Institution:** NORTH SHORE UNIVERSITY HOSPITAL MANHASSET, NY  
**Project Period:** 2002/07/01-2003/06/30

Gram negative bacterial infection is a widespread problem in critically ill patients. The high mortality of sepsis is in part mediated by bacterial endotoxin (LPS), which activates mitogen-activated protein (MAP) kinases (e.g., p38, ERK 1/2, and JNK), and stimulates the release of proinflammatory cytokines (e.g., TNF and IL-1beta), nitric oxide, platelet-activating factor, and other products. Macrophage-derived cytokines have been implicated in mediating lethal endotoxemia, because inhibition of their production or activity attenuates the development of tissue injury in animal models. If delivered early enough, anti-TNF can be an effective therapy in experimental models of endotoxemia, but early treatment is difficult to achieve in the clinic. An alternative strategy would be to identify "late" macrophage mediators that may be clinically more accessible. We recently identified a ubiquitous protein, HMGB1 (formerly known as HMG-1), as a late mediator of endotoxin lethality (Science 1999, 285: 248-251). HMGB1 is released late by LPS-stimulated macrophages, and its serum levels increase significantly between 16 to 32 hours after exposure to endotoxin. Anti-HMGB1 antibodies significantly protect against lethal endotoxemia and LPS-induced acute lung injury, even when antibody administration is delayed until after the early TNF response. Purified recombinant HMGB1 induced the release of multiple cytokines (e.g., TNF, IL-1beta and IL-6) in macrophage/monocyte cultures, and promoted tissue injury and even lethality when administered into mice. However, the mechanisms underlying the regulation of HMG-1 release and action are still unknown. The first aim of the studies outlined in this proposal is to determine the roles of early pro-inflammatory cytokines (e.g., TNF, IL-1beta) and MAP kinase (e.g., p38 and ERK1/2) signaling pathways in regulation of LPS-induced HMG-1 release in macrophage/monocyte cultures. This will be accomplished by examining the effect of TNF- or IL-1beta-specific neutralizing antibodies, as well as MAP kinase-specific inhibitors or anti-sense oligonucleotides on LPS-induced HMGB1 release. The second aim of this proposal is to examine the role of HMGB1 receptor (e.g., RAGE) and MAP kinases (e.g., p38, ERK1/2, and JNK) in regulation of HMGB1-induced cytokine production in macrophage/monocyte cultures. We will examine whether HMGB1 will activate MAP kinases, and whether RAGE-specific neutralizing antibodies or anti-sense oligonucleotides will prevent HMGB1-induced TNF release. Answers to these questions will shed light on the mechanisms underlying regulation of HMGB1 release and action, and improve our understanding of mechanisms underlying regulation of the innate immune response in endotoxemia.

**Grant:** 1R01GM063150-01A1  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** O'DOHERTY, GEORGE A PHD  
**Title:** Synthesis of Papulacandins A-D; New Antifungal Analogs  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2002/03/01-2002/08/31

Understanding how oligosaccharide structure controls these cellular recognition events has major implications for the treatment of many infectious diseases. Furthermore, understanding oligosaccharides structures and bio-synthesis are critical elements for discovering new chemotherapies. Due to the growing problem of bacterial resistance, there is a great need for new antibacterial/antifungal compounds. The project proposed herein is a tandem synthetic/biological investigation of the papulacandins, a class of potent mono-, di-, and trisaccharide antifungal antibiotics. Our study revolves around a unique de novo asymmetric synthetic approach to the papulacandin ring system, which should allow for easy access to these di and trisaccharides. In addition, this approach will allow simple access to unnatural analogs of this class of natural products and allow for easy screening for activity. Access to the papulacandin ring system and analogs will enable us to probe the mechanism of action to this antifungal agent and ultimately allow for the discovery of new more potent and stable antifungal agents. Additionally, these biological studies should also lead to new antibacterial, anticancer and antiviral compounds.

**Grant:** 1R01GM063821-01A1  
**Program Director:** OKITA, RICHARD T  
**Principal Investigator:** SVENSSON, CRAIG K  
**Title:** Mechanisms of Cutaneous Drug Reactions  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 2002/07/01-2005/06/30

DESCRIPTION (provided by applicant): Cutaneous drug reactions (CDR) are among the most frequent adverse medical events. Recent studies suggest that between 30 and 45 percent of all adverse drug reactions involve the skin. Of particular interest are the delayed-type hypersensitivity reactions that occur with sulfonamides and sulfones. While most investigators have focused on the role of differences in hepatic bioactivation and detoxification in determining predisposition to these reactions, it is uncertain and perhaps unlikely that liver-generated reactive metabolites would survive transit to the skin. We have developed a novel hypothesis wherein metabolic activation of drugs in keratinocytes provokes the release of signals that result in the activation of cutaneous dendritic cells, thereby initiating the cascade of events resulting in the manifestations of a CDR. The long term-goal of our project is to elucidate the mechanism of CDR and develop means to predict and/or prevent their occurrence. The objective of the present proposal is to test the validity of our hypothesis using sulfamethoxazole (SMX) and dapsone, which are among the most frequent CDR-inducing drugs, as model compounds. The Specific Aims of this project are to determine: 1) If cyclooxygenase is the enzyme that bioactivates SMX and dapsone in normal human epidermal keratinocytes (NHEK). Preliminary studies have indicated that these compounds can be bioactivated by cyclooxygenase-2. We will identify the enzyme responsible for this bioactivation in NHEK using selective inhibitors and inducers, as well as recombinant enzyme. 2) If cytokines alter the bioactivation or detoxification of SMX and dapsone in NHEK. An inflammatory response has been shown to alter enzymes important in drug bioactivation, an event that appears to be mediated by cytokines. Cytokines may also alter the glutathione content of cells, an alteration that may alter the susceptibility of cells to these hydroxylamine metabolites. We will assess the effects of proinflammatory cytokines on the bioactivation/detoxification of SMX and dapsone in NHEK. 3) If NHEK incubated with hydroxylamine metabolites of SMX and dapsone release signals resulting in the activation of dendritic cells. After determining the mechanism of cell death induced by these metabolites, we will test the hypothesis that they activate dendritic cells, either directly or indirectly (i.e., via signals released from NHEK). We anticipate that the results of the proposed studies will identify key points of intervention that will permit the prevention or management of these reactions. Moreover, they should enable us to develop in vitro screening tests that will permit the pre-clinical identification of drugs likely to pose a significant risk for the development of such reactions.

**Grant:** 1R01GM064463-01  
**Program Director:** ANDERSON, RICHARD A.  
**Principal Investigator:** ZUSMAN, DAVID R  
**Title:** Regulation of Sporulation in *Myxococcus xanthus*  
**Institution:** UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA  
**Project Period:** 2002/04/01-2006/03/31

The focus of this research project is the study of sporulation in the bacterium *Myxococcus xanthus* as cells form multicellular fruiting bodies. Starvation in *M. xanthus* triggers the developmental program, which causes cells to aggregate and sporulate. In this proposal, we plan to study the regulation of the timing of sporulation, as sporulation normally occurs in raised mounds after the completion of aggregation, but not in unaggregated cells. The delay in sporulation suggests that cells have a mechanism to monitor progress towards aggregation prior to triggering sporulation. The timing of sporulation is an interesting problem since sporulation marks the endpoint of a developmental program and the "decision" to follow that pathway is controlled by the microenvironment of the cells, which includes the spatial location of cells in reference to neighbor cells. The timing of developmentally regulated events in response to cell positioning is important for the proper development of all multicellular organisms, especially higher eukaryotes. We have recently discovered two genes that appear to be involved in the regulation of the timing of sporulation: *espA* and *espB*. Mutant phenotypes suggest that *EspA* inhibits sporulation until aggregation has been completed and *EspB* counters this inhibition. We propose to study the structure of *EspA* and how the individual domains modulate the negative regulation of downstream (sporulation) genes and upstream signaling genes. We plan to characterize these domains by preparing and analyzing mutants and studying the effects of the mutations on the functioning of the protein in regulating sporulation. We plan to follow the localization of *EspA* and *EspB* during development by cell fractionation studies, fluorescence microscopy, and construction and tracking of GFP fusions. The two Ser/Thr protein kinases encoded by genes flanking *espAB* will be studied for their possible roles in regulating the interaction between *EspA* and *EspB*. We plan to examine *EspA* and *EspB* for phosphorylation changes during development. In addition, we plan to characterize *HpkA*, a histidine protein kinase that regulates the expression of *EspA* and the substrate for the kinase. We also plan to search for the genes and gene products that are regulated by *EspA* - the downstream genes that initiate the sporulation process. The last part of the grant will explore the effect of C-signaling and high cell density in the control of sporulation.

**Grant:** 1R01GM064509-01A1  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** MORRIS, SIDNEY M PHD  
BIOCHEMISTRY:BIOCHEM  
RY-UNSPEC  
**Title:** Regulation of Arginase Genes in Sepsis and Trauma  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA  
PITTSBURGH  
**Project Period:** 2002/07/01-2006/06/30

Sepsis, traumatic injury, injury complicated by infection, and major surgery all initiate systemic responses that follow release of complex and varied combinations of cytokines and stress hormones, as well as of bacterial endotoxins in infection. In extreme conditions, these responses can result in death due to organ dysfunction and multiple organ failure. Major changes in arginine metabolism are major hallmarks of these pathophysiologic states. These changes include dramatic increases in expression of the arginases (isoforms I and II), which can have a significant impact not only on NO synthesis but also on synthesis of polyamines and proline, which are involved in cell proliferation and wound healing. We have shown that transcription of the arginase genes exhibits a complex pattern of responses to a wide variety of pro- and anti-inflammatory stimuli. It is particularly notable that responses of the arginases and iNOS to these stimuli are not identical. A clear understanding of the complex changes in arginine metabolism, as well as of the mechanisms underlying the responses to the complex combinations of pro- and anti-inflammatory cytokines and other agents, is essential for advances in the prevention or reversal of multiple organ failure. To elucidate these mechanisms, we propose the following aims: AIM I. To define the promoter elements and cognate transcription factors responsible for regulation of the arginase I gene-by pro- and anti-inflammatory agents. AIM II. To define the promoter elements and cognate transcription factors responsible for regulation of the arginase II gene by pro- and anti-inflammatory agents. AIM III. To define mechanisms involved in regulation of arginase II mRNA stability. In each of these aims, we will test specific hypotheses regarding transcriptional regulation of the arginase genes by individual stimuli and combinations thereof. Elucidation of the mechanisms involved in regulation of arginase gene expression, combined with independent efforts by others to elucidate the mechanisms involved in regulation of the iNOS gene, will greatly enhance our understanding of the molecular events underlying the pathophysiology of sepsis and trauma and thus aid in design of therapeutic strategies for these disease states.

**Grant:** 1R01GM064552-01  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** KILPATRICK, LAURIE E PHD  
**Title:** Apoptosis in Sepsis: Regulation by TNF & delta-PKC  
**Institution:** CHILDREN'S HOSPITAL OF PHILADELPHIA PHILADELPHIA, PA  
**Project Period:** 2002/01/10-2006/12/31

Spontaneous apoptosis of neutrophils is attenuated during sepsis and inflammatory cytokines such as TNFalpha have been implicated. The pathophysiological mechanisms involved in TNFalpha-mediated attenuation of apoptosis are poorly understood but proposed to be mediated by the p60 TNF receptor (p60TNFR). In adherent neutrophils to engage beta2 integrins, TNFalpha triggers phosphorylation of the p60TNFR, activation of cell survival signaling pathways and inhibition of spontaneous apoptosis. Our studies implicate phosphatidylinositol 3-kinase (PI 3-kinase) in TNFalpha triggered NFkappaB activation and that TNFalpha activation of PI 3-kinase requires beta2 integrins. The protein kinase C isotype delta (delta-PKC) phosphorylates p60TNFR on serine residue(s) in TNFalpha activated neutrophils. Rottlerin, a delta-PKC inhibitor, suppressed the inhibitory effect of TNFalpha on neutrophil apoptosis and activation of the transcription factor NFkappaB suggesting that delta-PKC may regulate TNFalpha anti-apoptotic signaling. Our model proposes a selective role for delta-PKC in regulating anti-apoptotic signaling triggered by TNFalpha binding to the p60TNFR. The goal of this study is to establish a role for delta-PKC in TNFalpha mediated anti-apoptotic signaling through the p60TNFR using antisense technology to selectively deplete delta-PKC from HL60 cells differentiated to a neutrophilic phenotype. We will: 1: Determine whether delta-PKC regulates TNFalpha mediated anti- apoptotic signaling. Determine the effect of delta-PKC deletion on TNFalpha-mediated suppression of spontaneous apoptosis and activation of the antiapoptotic NFkappaB and the MAP kinases ERK1/2. 2: Assess the role of PI 3-kinase in TNFalpha mediated suppression of spontaneous apoptosis, and determine if delta-PKC is required for TNFalpha mediated activation of PI 3-kinase. 3: Identify the delta-PKC phosphorylation site on the p60TNFR and establish if phosphorylation is in the a) death domain, b) juxtamembrane region, or c) a novel domain. 4: Assess the role of delta-PKC in regulating the assembly of TNFalpha anti-apoptotic signaling complexes and their association with the p60TNFR.



**Grant:** 1R01GM064576-01  
**Program Director:** IKEDA, RICHARD A.  
**Principal Investigator:** LIWANG, ANDY C BS  
**Title:** Tertiary Structures of Circadian Clock Proteins by NMR  
**Institution:** TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX  
**Project Period:** 2002/04/01-2007/03/31

**DESCRIPTION:** (provided by applicant) The physiology and behavior of virtually all organisms oscillate with a periodicity of approximately 24 h, which is now known to be the result of an endogenous oscillator called the circadian clock. All circadian clocks studied thus far are based on a common design composed of a transcription/translation feedback loop of clock genes and clock proteins. The activity of a clock protein is modulated by its interactions with other clock proteins and these protein-protein interactions play an important role in the periodicity of the circadian rhythm. As no high-resolution structure of a circadian clock protein has been solved, no firm structural predictions can be made as to how clock proteins interact and, therefore, how their interactions help achieve a normal (or abnormal) circadian periodicity of the oscillator. As a result, the mechanism of the circadian pacemaker at the structural level is still unclear. It is proposed here that significant advances in understanding the structural basis of circadian time keeping can be achieved by investigating the three-dimensional structures and dynamics of the clock proteins of *Synechococcus elongatus*, SasA, KaiA, KaiB and KaiC using nuclear magnetic resonance spectroscopy (NMR). The specific aims are to solve the structures of these clock proteins by NMR and then elucidate the protein-protein interactions central to setting the pace of the circadian rhythm. The data base of genetic and biochemical information on the function of the circadian clock of *S. elongatus* is quite extensive and, if mapped onto the three dimensional architecture of the circadian clock, will allow insights into the molecular mechanism of a circadian pacemaker. For example, whether residues E103, R249 and E274 of KaiA, whose mutation alter the circadian rhythm, are clustered together on the surface or make important hydrogen bonds in the core of the protein, are important questions of the research proposed here. If the residues are clustered together on the surface, NMR studies will ascertain whether they form part of the dimer interface with KaiB, KaiC, and/or SasA. If the residues are buried in the core of the protein, comparisons of the E103K, R249H and E274H mutants with wild-type KaiA will reveal aspects of the structures and dynamics that are important to the function of KaiA. The long-range objective is to eventually investigate clock protein complexes by NMR in an effort to understand how specific protein-protein interactions modulate circadian rhythms. Key questions of our long-range objectives are whether interactions between KaiA, KaiB, SasA, and KaiC involve allostery, localized changes in backbone or side chain dynamics, displacement of tightly bound water molecules or rearrangement of crucial hydrogen bonds, and how they play important roles in setting the pace of the circadian rhythm. Although some of the conclusions resulting from the work proposed here will be specific to *S. elongatus*, others, should have universal ramifications for all organisms, including humans.

**Grant:** 1R01GM064600-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** CROSA, JORGE H PHD  
CHEMISTRY:CHEMISTRY-  
UNSPEC  
**Title:** Assembly Line Biosynthesis of Bacterial Siderophores  
**Institution:** OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR  
**Project Period:** 2002/02/01-2006/01/31

DESCRIPTION (provided by the applicant): The goal of this research is to understand the mechanism of biosynthesis of bacterial peptide siderophores. We use as a paradigm the peptide siderophore anguibactin that is produced by the pathogenic bacterium *Vibrio anguillarum*. Anguibactin is an important component of the pJM1 plasmid-mediated iron uptake system that is essential for virulence of these pathogenic vibrios. Genetic and physiological analysis led us to the identification and cloning of genes encoded on the pJM1 plasmid that play an essential role in anguibactin biosynthesis. DNA sequence and protein analysis revealed that these genes encode polypeptides that possess domains found in nonribosomal peptide synthetases (NRPSs), originally identified as components of the biosynthetic machinery for the synthesis of antibiotics in gram-positive bacteria. These proteins have been named AngB, AngM, AngN, and AngR and possess modules that could be involved in one or more of the following reactions during the biosynthesis of anguibactin: peptidyl carrier protein (PCP), involved in thioester formation; condensation (C), intervening in peptide bond formation; cyclization (Cy), involved in both condensation and heterocycle formation, and adenylation (A), which is responsible for substrate activation. AngB is an isochorismate lyase that also operates as an aryl carrier (ArCP) protein during siderophore assembly. Other proteins encoded by plasmid-mediated genes, such as AngH and AngU, possess enzymatic activity for the synthesis of histamine from histidine, and for the further oxidation of this compound to hydroxy-histamine, which is a basic building block of anguibactin. Our present efforts are thus directed to elucidate the role of the specific modules of the NRPSs in siderophore biosynthesis. The specific aims to achieve this goal are: 1. Dissection of the mechanisms of assembly line enzymology of anguibactin biosynthesis. We have purified these NRPSs proteins and have obtained antibodies which will be used in the assessment of the role of these polypeptides by using in vitro synthesis reactions including swapping of equivalent NRPS modules intervening in siderophore biosynthesis in *V. anguillarum*, *Vibrio cholerae*, and other pathogens. 2. Mutational analysis of the NRPSs genes that will include random mutagenesis and site-directed mutagenesis of the specific modules. We will also purify selected mutant proteins to be used in in vitro synthesis reactions to identify single steps during anguibactin assembly. 3. Identification of chromosomal-encoded proteins intervening in anguibactin biosynthesis. We will use a combination of transposon-directed cloning, genetic, immunological and biochemical approaches to characterize these genes. The combination of the in vivo genetic and the in vitro biochemical approaches will likely lead to the dissection of the mechanisms of siderophore biosynthesis and, in turn, to the exploration of new avenues to understand this contribution to bacterial virulence.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R01GM064640-01  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** MOORMAN, J RANDALL MD  
**Title:** Heart rate characteristics monitoring in newborn infants  
**Institution:** UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA  
CHARLOTTESVILLE  
**Project Period:** 2002/03/01-2005/02/28

DESCRIPTION (provided by applicant): Sepsis is a major health problem in high-risk newborn infants in the neonatal intensive care unit (NICU), where it occurs in 25 percent of very low birth weight infants and leads to a more than doubling of mortality and a 50 percent increase in hospital stay. Our long-term objective is to test the hypothesis that detection of abnormal heart rate characteristics (HRC) with continuous non-invasive monitoring will improve care of these patients by earlier diagnosis of sepsis and other sub-acute, potentially catastrophic illnesses. We propose to advance toward this objective by completing two research aims. In Aim 1, we will prospectively study unselected infants in a university NICU to test the hypotheses that abnormal HRC will be associated with upcoming sepsis and sepsis-like illness as defined by objective illness criteria, and that HRC adds significant independent diagnostic information about the risk of sepsis and sepsis-like illness to clinical variables of birth weight and gestational age. The clinical research design is for derivation of multivariable predictive statistical models from one set of patients followed by a validation phase using clinical data from a second set of patients. In Aim 2, we will develop new measures of HRC that are specific to the task of early detection of neonatal sepsis. We will investigate optimum choice of parameters for sample entropy calculation, stationarity of heart rate time series using conventional and novel measures based on the empirical cumulative distribution function, and frequency domain analysis using a novel method.

**Grant:** 1R01GM064671-01A1  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** LEVIN, PETRA A  
**Title:** Temporal and Spatial Control of B subtilis cytokinesis  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2002/07/01-2007/06/30

DESCRIPTION (provided by applicant): How cells determine when and where to divide remains one of the great mysteries of modern biology. Spatially, division is tightly regulated to ensure the accurate positioning of septa. Temporally, division is coordinated with DNA replication and chromosome segregation. From bacteria to yeast to humans, cell division is initiated by the formation of a ring of a cytoskeletal protein at the nascent division site. This ring establishes the location of the division septum and serves as a framework for assembly of the division apparatus. In bacteria this ring is composed of the essential tubulin-like GTPase FtsZ. This proposal focuses on the regulatory networks that govern FtsZ ring formation in the soil bacterium *B. subtilis*. The factors that establish the division site and couple FtsZ ring formation to the cell cycle remain unknown. Comprehending the spatial and temporal regulation of bacterial division thus requires the identification of the cellular and molecular mechanisms that stimulate FtsZ ring formation at midcell in response to cell cycle cues and inhibit FtsZ ring formation at all other sites. EzrA, a factor that helps restrict FtsZ ring formation to midcell, was identified through classical genetic screens. Preliminary biochemical data suggest that EzrA interacts directly with FtsZ to inhibit ring formation by destabilization of FtsZ polymers. This proposal has three major goals. One, to characterize the molecular mechanism by which EzrA prevents ectopic FtsZ ring formation. Two, to clone and to characterize the gene identified by *wee2*, a mutation that uncouples cell division from growth, leading to the formation of small cells, many less than 25% the size of wild type *B. subtilis*. Three, to extend genetic screens to identify additional factors that (i) promote FtsZ ring formation at midcell, (ii) couple FtsZ ring formation to the cell cycle, and (iii) inhibit FtsZ ring formation at inappropriate sites. As essential components of the bacterial cell division machinery, FtsZ and the factors governing its activity hold promise as potential targets for the development of new antibiotics. Furthermore, this work should illuminate not only bacterial cell division, but also aspects of cytokinesis fundamental to all organisms. Understanding the molecular mechanisms that normally control cell division will help identify why they fail during oncogenesis, leading to the aberrant divisions and rapid proliferation characteristic of cancer cells.

**Grant:** 1R01GM064713-01  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** BRAY, DENNIS DOTH ZOOLOGY AND  
CHEMISTRY  
**Title:** MOLECULAR EVENTS IN A DISCRETE CYTOPLASMIC SPACE  
**Institution:** UNIVERSITY OF CAMBRIDGE CB2 1TS,  
**Project Period:** 2002/06/01-2005/05/31

DESCRIPTION (provided by applicant): In this project, a small and specialized volume of cytoplasm associated with a cluster of chemotactic receptors on the surface of the bacterium *Escherichia coli* will be analyzed in great detail, using computer modeling, stochastic simulation techniques, and a novel 3D prototyping technique. Models of the receptors and associated proteins, at atomic resolution, will be assembled into a two-dimensional lattice resembling the cluster of receptors in the bacterial membrane in an arrangement consistent with biochemical, kinetic, mutational and behavioral data. The cytoplasmic domains of the receptors will then be used to define a small volume of cytoplasm subjacent to the plasma membrane and to examine the concentrations, distributions and diffusive behavior of "soluble" enzymes that interact with the receptors in the course of adaptation. The feasibility of a two-handed, "brachiation" motion of enzymes through the lattice, due to their possession of two relatively weak binding sites for the receptors will be examined. We will also explore the theoretical possibility that conformational changes might spread, from one receptor to its neighbors in the lattice via intervening proteins, and thereby lead to the emergence of large-scale coherent patterns of activity. The results of this study are likely to provide novel insight not only on bacterial chemotaxis but also, more generally, on other membrane-associated protein complexes, such as focal adhesions in fibroblasts and postsynaptic densities in the vertebrate central nervous system.

**Grant:** 1R01GM065113-01  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** KUO, PAUL C MD  
**Title:** NO induces osteopontin, a potent trans-repressor of iNOS  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2002/07/01-2006/06/30

In endotoxin (LPS)-mediated sepsis, inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production alter multiple functions, including cardiac contractility, vasomotor tone, intestinal epithelial permeability, and leukocyte recruitment. While the molecular pathways which upregulate iNOS in endotoxemia have been extensively characterized, little is known of the corresponding pathways which downregulate iNOS. Utilizing both in vivo murine and in vitro murine macrophage and rat hepatocyte models of LPS stimulation, we have demonstrated that NO feedback inhibits its own synthesis by increasing gene transcription and promoter activation of osteopontin (OPN), a potent trans-repressor of iNOS expression. This negative feedback pathway of NO-dependent OPN gene transcription and protein synthesis has not been previously described. We hypothesize that transcription of OPN, a potent trans-repressor of iNOS expression, is NO-dependent in LPS-stimulated murine macrophages. In the immortalized ANA-1 murine macrophage cell line, we propose to functionally map the OPN promoter in the context of LPS stimulated NO production. Specific Aim 1. To define NO-dependent cis-acting transcriptional control regions, we will utilize OPN promoter-reporter constructs with deletion analysis and site-directed mutagenesis. Specific Aim 2. To define NO-dependent trans-acting regulation, we will isolate the NO-dependent transcription factor and its cDNA clone by using the biotin-streptavidin affinity method and screening a cDNA expression library, respectively, using the DNA recognition site as a probe. Specific Aim 3. To determine the S-nitrosylation status of our transcription factor and its effect upon DNA binding, we will use CuCl-cysteine coupled chemiluminescence. Specific Aim 4. To confirm relevancy in the LPS-stimulated macrophage, we will inhibit translation of the transcription factor mRNA with antisense techniques and alternatively, over-express the transcription factor by transient transfection. Specific Aim 5. To confirm in vivo relevancy, we will utilize a murine OPN-knockout model of endotoxemia to demonstrate lack of iNOS inhibition in the absence of OPN. Our studies will define OPN production as a unique and as yet, poorly characterized, NO-dependent transcriptional pathway which inhibits iNOS expression in the setting of endotoxemia.

**Grant:** 1R01GM065175-01  
**Program Director:** WOLFE, PAUL B.  
**Principal Investigator:** FOSTER, PATRICIA L PHD  
**Title:** Regulation of an Error-Prone Polymerase  
**Institution:** INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN  
**Project Period:** 2002/04/01-2006/03/31

Genomic stability is vital to the health of the individual and the preservation of the species. This stability can be threatened by DNA damage from endogenous and exogenous sources. However, mutations, which are heritable sequence changes in the DNA, can also arise as a result of errors made during replication of undamaged DNA. Recently an entirely new family of error-prone DNA polymerases has been discovered. Found in all three domains of life, these polymerases are beneficial because they can replicate past DNA lesions, but they are also potentially detrimental because they make frequent errors, even on undamaged DNA. If the activities of these polymerases are not controlled, they could be a potent source of the mutations that lead to genetic disorders such as cancer. The hypothesis underlying the proposed research is that cells can and must control the activities of their error-prone DNA polymerases. To test this hypothesis, pathways that control the activity of *E. coli*'s error-prone DNA polymerase IV (Pol IV) will be found and characterized. The specific aims are: (1) to identify new regulatory factors that affect the mutagenic activity of Pol IV; (2) to characterize the proteins and pathways regulating Pol IV levels or activity; and, (3) to further characterize the regulatory factors that are already identified. Because Pol IV is a close homologue of eukaryotic error-prone DNA polymerases, higher organisms, including humans, may use similar control mechanisms.



**Grant:** 1R01GM065180-01  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** LITTLE, JOHN W PHD  
**Title:** Biochemical and structural analysis of LexA cleavage  
**Institution:** UNIVERSITY OF ARIZONA TUCSON, AZ  
**Project Period:** 2002/04/01-2006/03/31

DESCRIPTION: (provided by applicant): LexA repressor of E. coli plays a central role in regulation of the SOS response to DNA damage. LexA represses a set of about 20 genes during normal growth. After DNA damage, RecA protein is activated to a form that mediates cleavage of LexA. Cleavage inactivates LexA, leading to derepression of the SOS regulon. The basis for the proposed work is the crystal structures of several mutant LexA proteins. Two forms of LexA are observed. In the NC form, the cleavage site is distant from the active site. In the C form, the cleavage site lies in the active site and is adjacent to the nucleophile that attacks the peptide bond. It is proposed to use the structures as a guide for further analysis of several LexA functions. First, the model will be tested that these forms represent the mechanism controlling reactivity of LexA. Mutants will be made that are predicted to stabilize the C form, and their cleavage rates will be tested. Interesting proteins will be analyzed by x-ray crystallography. Second, dimerization mutants will be made and characterized. Third, in order to characterize further the chemical mechanism of LexA cleavage, efforts will be made to develop a simple substrate for this reaction, and to isolate a covalent intermediate. Fourth, the model will be tested that other cleavable proteins in the LexA superfamily, notably UmuD and lambda repressor, have the same mechanism for controlling their cleavage reactions. Fifth, the structure will be used as a guide to identify portions of LexA involved in interaction with activated RecA. RecA-mediated cleavage will be analyzed in more detail. Efforts will be made to solve the structure of a RecA:LexA complex.

**Grant:** 1R01GM065243-01  
**Program Director:** JONES, WARREN  
**Principal Investigator:** ZHANG, HONG BS  
**Title:** Crystallographic Analysis of NMN Adenylyltransferases  
**Institution:** UNIVERSITY OF TEXAS SW MED DALLAS, TX  
CTR/DALLAS  
**Project Period:** 2002/06/15-2006/05/31

**DESCRIPTION:** (provided by applicant) Nicotinamide mononucleotide adenylyltransferase (NMNAT) is an indispensable enzyme in the biosynthesis and salvage of NAD and NADP in all living organisms. In prokaryotes, it is absolutely required for cell survival, thus representing an attractive target for designing new broad-spectrum anti-infectious pharmaceuticals. There is also considerable medical interest in human NMNAT because it catalyzes the key step in the metabolic conversion of the antitumor drug tiazofurin to its active form, tiazofurin adenine dinucleotide (TAD). Because of the vital roles of NAD in the cell, adequate levels of NAD must be maintained. In bacteria, a single protein NadR performs multiple functions in response to the cellular NAD and ATP levels. These include a transcriptional repressor, a NMN adenylyltransferase, and a nicotinamide ribose kinase. NadR regulates the expression of the genes involved in both de novo biosynthesis and salvage of NAD. It also controls the uptake of NMN and its subsequent adenylation. Structural information of NMNAT and NadR, along with their complexes with substrates will be essential for understanding the catalysis, substrate recognition, inhibition, as well as regulation of these important enzymes. We have solved the crystal structure of the first bacterial NMNAT in its ligand free form and have obtained well diffracting human NMNAT crystals. Comparison of the bacterial and human enzyme structures will help to design specific bacterial inhibitors with high selectivity. Additionally, we have expressed and purified NadR proteins from *S. typhimurium* and *H. influenzae*, and the crystallization trials are in progress. Elucidating the structures of NadR in its various ligand-bound forms and its complex with DNA will reveal how its conformation and function are modulated by both NAD and ATP.

**Grant:** 1R01GM065319-01  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** KARZAI, ABDUL W PHD  
**Title:** Quality Control of Protein Translation  
**Institution:** STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY  
**Project Period:** 2002/04/01-2007/03/31

**DESCRIPTION:** (provided by applicant): Bacteria possess a unique system for rescuing aberrantly stalled ribosomes and marking for degradation the still linked, partially synthesized protein fragments. This quality control system, also known as transtranslation, is orchestrated by a remarkable RNA (SsrA RNA) that functions as a tRNA to detect and revive stalled ribosomes and as an mRNA to facilitate the addition of a short degradation tag to the C-terminus of nascent polypeptides. All known activities of SsrA require SmpB, a small protein that binds SsrA specifically and with high affinity to promote its association with stalled ribosomes. The molecular basis for the formation of the SmpB-SsrA complex and the subsequent recognition of impaired ribosomes are not well understood. The objective of this research program is to use a combination of molecular genetics, protein biochemistry, bioinformatics, and structural approaches to elucidate the mechanism of the SmpB-SsrA quality control system. The emphasis is on the molecular characterization of how SmpB recognizes SsrA RNA and promotes the detection and rescue of stalled ribosomes. Principally, through these studies we wish to understand the biochemical and structural basis for the interactions of SmpB with SsrA RNA. Specifically we want to learn what amino acid residues are involved, what base-specific contacts are made, and what structural features contribute to the formation of the SmpB-SsrA complex and its interaction with the ribosome. Furthermore, we wish to identify and characterize any additional cellular factors that might participate in this process. Specific complexes of RNA and protein perform many essential biological functions, including RNA processing, RNA turnover, RNA transport, RNA folding, as well as the translation of genetic information from mRNA into protein sequences. Principles that govern RNA-protein interactions are inadequately understood due in large part to a paucity of structural information on RNA-protein complexes. These principles are important for understanding RNA-protein machines, such as the ribosome, and RNA-protein structure and function in general. The relative simplicity of the SmpB-SsrA interaction, the stability of the complex, and recruitment of additional novel factors during trans-translation makes it an ideal system to study the basic principles underlying the assembly of RNA-protein complexes. Understanding of the RNA-protein assembly processes in this system are likely to provide new insights generalizable to the molecular mechanism of how RNA-binding proteins function. Moreover, because the SmpB-SsrA quality control system exists only in prokaryotes and involves novel RNA and protein factors that are essential for the survival of most (if not all) pathogenic bacteria, a better understanding of this unique process might allow the design of highly specific new anti-bacterial agents.

**Grant:** 1R01GM065324-01  
**Program Director:** IKEDA, RICHARD A.  
**Principal Investigator:** WHITMAN, CHRISTIAN P  
**Title:** Structure and Mechanism in the Tautomerase Superfamily  
**Institution:** UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX  
**Project Period:** 2002/04/01-2006/03/31

DESCRIPTION (provided by applicant): The tautomerase superfamily consists of structurally homologous enzymes based on a beta-alpha-beta motif whose members use Pro-i as the general base in tautomerization and isomerization reactions. The long-term goal of this research is to determine the molecular and structural basis for catalysis and specificity in the tautomerase superfamily. This research has implications for our understanding of fundamental enzymatic reactions and the evolution of enzymes. In addition, these studies will assist in determining the range of metabolic capabilities for various pathogenic organisms, potentially leading to the development of new drugs, and facilitate bio-remediation efforts. The focus of this application will be representative members of the 4-oxalocrotonate tautomerase (4-OT) family. These enzymes range in size from 61-79 amino acids per monomer and all have an amino-terminal proline. The principal investigator's group has recently discovered structural and mechanistic diversity in this family. This diversity suggests that Nature used these short sequences (encoding a simple beta-alpha-beta motif) to create new structures and activities. The major specific aims will be to determine the mechanisms and structures for 1) 3-chloroacrylic acid dehalogenase which may use Pro-1 to activate water for an addition reaction, 2) malonate semialdehyde decarboxylase, which may use Pro-1 in a Schiff base mechanism to facilitate decarboxylation, 3) a tautomerase, which has low level isomerase and dehalogenase activities, 4) a dimeric 4-OT homologue, and 5) two closely related homologues that have tautomerase and isomerase activities but lack the conserved active site residues (except Pro-1) found in the parent member, 4-OT. Finally, experiments are proposed to improve the low-level activities and to manipulate the oligomer state by rational design. These studies will provide a better understanding of the structure/function relationships for each enzyme. A comparison of the strategies and active site structures will provide signatures for each enzymatic activity, shed light on how these activities evolved, and assist in the assignment of function. As a result, the underlying principles used in this system will be identified so that Nature's processes could be mimicked to create new activities and structures using the beta-a-beta motif.

**Grant:** 1R01GM065470-01  
**Program Director:** JONES, WARREN  
**Principal Investigator:** KAO, CAMILLA M PHD  
**Title:** DNA MICROARRAY ANALYSIS OF ENGINEERED NATURAL PRODUCTS  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 2002/03/01-2006/02/28

DESCRIPTION (provided by applicant): We are using DNA microarrays to study the *Streptomyces* bacteria, which have been an extremely rich source of antibiotics and other therapeutics for the pharmaceutical industry. In 1993, the antibiotic production capability of *Streptomyces coelicolor* was harnessed at Stanford University in the design of a versatile production system for engineered natural products. This production system has since been used to generate over one hundred novel natural product-like molecules, demonstrating new strategies for developing therapeutics against antibiotic-resistant pathogens and other medical diseases. However, many of these molecules are produced in small quantities in vivo, and future challenges are aimed at improving production yields that are commensurate with biological screening assays and commercial distribution. Recently, scientists at KOSAN Biosciences (Hayward, CA) isolated *S. coelicolor* production strains that generate more than ten-fold greater yields of engineered natural products. Increased production was found to be associated with the presence of abnormally high copy numbers of the production plasmid encoding antibiotic synthesis genes. This phenotype is now being harnessed as a tool by KOSAN to generate other high-producing strains, and the company is attempting to identify the genetic changes that alter copy number regulation. As a collaboration between Dr. Camilla Kao at Stanford University and Dr. C. Richard Hutchinson at KOSAN Biosciences, we are also attempting to understand the metabolic differences between production strains with high and low titers, and which genetic elements in the production plasmids contribute to increased product yields when present at high copy number. Greater understanding of the metabolic and genetic factors that influence natural product production should lead to new multi-pronged strategies for improving *Streptomyces* and other microbial production systems.

**Grant:** 1R01GM065471-01  
**Program Director:** JONES, WARREN  
**Principal Investigator:** SCHMIDT-DANNERT, CLAUDIA PHD  
**Title:** Biosynthesis of Unnatural Porphyrins in E. Coli  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2002/01/10-2005/12/31

The proposed research seeks to combine techniques of metabolic engineering with those of directed enzyme evolution (termed 'molecular pathway breeding') for the protection of useful novel chemical compounds and materials in recombinant cells. This application seek so to expand this approach to the biosynthesis of yet other, even more complex novel chemical compounds-porphyrins-demanding the development of new screening and analytical tools. Porphyrins are a fascinating, structurally complex compound class of enormous significance and potential for a variety of applications in e.g. medicine, chemistry or material sciences. Mimicking natural breeding processes through gene assembly and in vitro evolution of only a few functionally diverse available microbial porphyrin biosynthetic genes will generate new pathways for unnatural porphyrins. The strategies and methods developed in this project can be readily applied to other metabolic pathways and provide new engineering tools for the discovery and production, in recombinant cells, of a kaleidoscope of diverse natural and non-natural compounds for a variety of applications. In particular, the developed high-throughput (HT) screening methods for the identification of synthesized small molecules in large cell libraries will be highly useful for other combinatorial approaches as well. The biosynthetically produced new porphyrin structures with various reactive functional groups will make valuable scaffolds for chemical modifications for the synthesis of additional porphyrin structures.

**Grant:** 1R01GM065546-01  
**Program Director:** IKEDA, RICHARD A.  
**Principal Investigator:** TANNER, JOHN J PHD  
**Title:** Structural Studies of the Multifunctional PutA Protein  
**Institution:** UNIVERSITY OF MISSOURI COLUMBIA COLUMBIA, MO  
**Project Period:** 2002/05/05-2006/04/30

DESCRIPTION (provided by applicant): The goal of this project is to characterize structure-function relationships for the multifunctional flavoprotein, PutA from Escherichia coli. This remarkable protein is both a transcriptional repressor of the proline utilization (put) regulon and a membrane-associated proline catabolic enzyme. The three-dimensional structural basis for the versatility of PutA is unknown. The working hypothesis whereby PutA changes its intracellular location and function is that conformational changes governed by the flavin redox state control its macromolecular associations (i.e. DNA and membrane-binding). The proposed research addresses three fundamental outstanding questions related to PutA structure and function: (1) What is the three-dimensional structure of PutA? (2) How does PutA interact with DNA? and (3) What are the conformational changes that allow PutA to function as both a DNA-binding protein and a membrane bound enzyme? The first aim of this proposal is to determine the three-dimensional structure of PutA using X-ray crystallography. Crystallization of PutA is challenging due to its large size (1320 amino acid residues) therefore a "divide and conquer" strategy will be employed in which shorter polypeptides that retain one or more of the functions of PutA will be engineered and crystallized separately. These smaller structures will then be stitched together computationally to derive a model of the full-length protein. Good progress has already been made using this approach - the 2.0 Å crystal structure of a protein corresponding to the first 669 residues of PutA has been solved. The second aim is to determine the structural basis for PutA-DNA interactions by solving the crystal structures of PutA and truncated PutA proteins complexed with well-defined DNA binding sites. The third aim is to explore the conformational changes induced by proline reduction of the flavin by determining the crystal structures of PutA and truncated PutA proteins in the proline-reduced state. These studies will contribute pivotal understanding into the regulatory mechanism of PutA and timely knowledge of its structure.

**Grant:** 1R01GM065891-01  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** ROBERTS, GARY P  
**Title:** PII: multiple homologs of a global regulator  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2002/09/01-2006/08/31

DESCRIPTION (provided by applicant): The members of the PII (GlnB) protein family are among the most broadly distributed regulatory proteins and are primarily involved in central nitrogen regulation in bacteria, archaea and in some plants. This protein family is biologically important not only for its distribution, but also for the critical roles that it has been shown to play in all aspects of nitrogen regulation and the balancing of carbon and nitrogen utilization. In the past few years, three very significant developments have occurred that this proposal seeks to take advantage of: The crystal structures of two members of this family have been solved, many organisms have been shown to have more than one homolog, and homologs in different organisms have been implicated in rather different biochemical functions. A critical issue, and a focus of this proposal, is the molecular basis of these different biochemical roles. This proposal will address this issue using our recent observation that *Rhodospirillum rubrum* has three homologs (named GlnB, GlnJ, and GlnK), with rather similar sequences, but with at least four different readily assayed functions *in vivo*. This provides an excellent model system for identifying functionally important residues in these homologs, correlating them with specific biological roles and eventually determining the specific receptor proteins that interact with the PII (GlnB) homologs in these roles. Identification of the critical regions of the PII homologs involved in different protein interactions will be of biological importance, especially when coupled with a better understanding of the proteins with which they interact. We cannot understand the diverse metabolic roles of the PII family until we have precisely this information. Only such information will, for example, explain the role of the post-translational uridylylation that is common among PII homologs. Given the extreme conservation of PII sequence across much of biology, it is a reasonable hypothesis that critical surfaces involved in the interactions of the PII homologs of *R. rubrum* will serve as important regions in other PII homologs as well.



**Grant:** 1R01GM065934-01  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** MEINERS, JENS-CHRISTIAN PHD  
**Title:** Mechanics of Transcriptional Control through DNA Looping  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/09/01-2007/08/31

DESCRIPTION (provided by applicant): The overall long-range objective is to quantitatively understand the mechanics of transcriptional control through DNA looping. This will not only elucidate the relationship between the structure, dynamics and function of such regulatory loops, but also improve our understanding of a wide range of fundamental life processes in which proteins interact with multiple sites on a DNA molecule, such as site-specific recombination and the regulation of replication. For this aim, a simplified model system for DNA looping based on the lac repressor and the lac operon in *E. coli* will be studied in vitro in a series of single-molecule experiments. Specifically, state-of-the-art single-molecule techniques, such as an optical-tweezer based femtoNewton force spectroscopy technique and total-internal-reflection fluorescence microscopy methods will be adapted and further improved to allow the measurement of forces that are associated with the loop formation process with femtoNewton sensitivity and millisecond time resolution while the substrate DNA is subjected to mechanical constraints that are ubiquitous in a living cell, such as tension, twist and supercoiling. The dependence of protein-mediated loop formation rates on these mechanical features will be studied, and quantitatively interpreted in the framework of statistical mechanics of DNA molecules. In the end, it will be attempted to directly control transcription by mechanically opening and closing the repressor loop through the application of tension. This will test current models of how DNA looping regulates transcription and explore the role of mechanical constraints on this important process. This will help to bridge the gap between oversimplified models for DNA looping such as in-vitro DNA ring cyclization, and transcriptional repression in a living cell, which is currently inaccessible to a quantitatively accurate theoretical description. On the side, the instrument development efforts will pave the way for a multitude of other ultra-sensitive single-molecules studies in fields as diverse as the dynamics of protein- and RNA folding or improved studies of molecular motors.

**Grant:** 1R01GM066011-01  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** RICE, PHOEBE A BA  
**Title:** Understanding DNA specificity in the IHF/HU family  
**Institution:** UNIVERSITY OF CHICAGO CHICAGO, IL  
**Project Period:** 2002/08/15-2006/07/31

DESCRIPTION (provided by applicant): These studies have two overall goals: First, to further our understanding of protein-DNA interactions, particularly the role of DNA structure in site recognition, and second, to better understand the biological roles of the highly conserved HU/IHF family of proteins that have been selected for detailed study. Recognition of particular DNA sites by proteins is the sine qua non for the regulation of gene expression and many other biological processes. In some cases, such recognition is a straightforward consequence of hydrogen bonds between properly arrayed protein side chains and the edges of bases in the major groove of B-form DNA. In many instances, however, the situation is much more complicated, and recognition depends on sequence - dependent variations in the structure and distortability of the DNA ("indirect readout"). This form of recognition is not as well understood as the former. The IHF/HU family proteins are small, closely related prokaryotic DNA bending proteins that function as architectural factors in a variety of processes (e.g. transcription and recombination) that require multicomponent protein-DNA complexes. Two of the proteins to be studied, IHF and Hbb, recognize specific (yet different) sequences in the DNA, but do so almost entirely through indirect readout. The third, HU, binds nearly independently of DNA sequence but recognizes specific structural distortions in DNA, and may play a role in DNA repair. It is well established that IHF binding introduces a nearly 180 degree bend in the DNA, but estimates of the bend introduced by HU vary widely. This uncertainty hampers our understanding of how HU functions in conjunction with other proteins in the cell. This project will study the binding properties of these proteins both in solution and by x-ray crystallography. Hypotheses based on comparing and contrasting such data for the 3 different proteins to be studied will be tested by site-directed mutagenesis and domain-swap experiments.

**Grant:** 1R01GM066014-01  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** ERICKSON, HAROLD P  
**Title:** Structure and Assembly Dynamics of FtsZ  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2002/07/01-2006/06/30

DESCRIPTION (provided by applicant): FtsZ, a homolog of tubulin, is the major cytoskeletal protein of bacterial cell division. FtsZ forms a ring around the center of the bacterium, which remains in place for most of the cell cycle. Ultimately the Z-ring constricts to divide the cell. In vitro, FtsZ assembles into long, straight protofilaments (pfs) that can associate into pf pairs and sheets. We have recently proposed a model in which FtsZ pfs assemble isodesmically, which is very different from the cooperative assembly of actin and microtubules. This model predicts a rapid fragmentation and annealing of pfs coupled to GTP hydrolysis. Using fluorescence recovery after photobleaching (FRAP) for in vivo analysis, we have also determined that FtsZ in the Z-ring is turning over rapidly, with a 30 sec halftime. This is consistent with our expectations from the isodesmic assembly model. However, that model at present is based largely on predictions from pf length, and it is essential to obtain experimental data confirming or modifying it. Most important is a direct measure of the interaction affinity of FtsZ subunits in the pf. Is it on the order of nM as predicted by the theory, or on the order of uM as predicted by other observations? We propose to use fluorescence anisotropy to measure the association of labeled subunits to pfs and estimate the Kd. We will then use the Biacore for more quantitative analysis, to determine the Kd for pf assembly, and hopefully the length of pfs assembled at different FtsZ concentrations. For several studies we will produce cap mutants that are blocked for assembly at one or the other ends. Their association into heterodimers should be a much simpler reaction than assembly of full pfs. The cap mutants will also be used to study the GTPase mechanism in vitro. Complementing these in vitro studies, we will extend our FRAP study of in vivo dynamics to new FtsZ mutants and accessory proteins. An important question that we can now address by FRAP is the state of assembly of FtsZ in the bacterial cytoplasm - is it monomers or pfs? We will determine this by diffusion measurements. Overall, we are aiming for a complete characterization of the biophysics of FtsZ protofilament assembly in vitro, and complementary analysis by FRAP of assembly dynamics and function in vivo.

**Grant:** 1R01GM066072-01  
**Program Director:** OKITA, RICHARD T  
**Principal Investigator:** EVANS, MARTIN E MD  
**Title:** Optimizing dosing to prevent antibiotic resistance  
**Institution:** UNIVERSITY OF KENTUCKY LEXINGTON, KY  
**Project Period:** 2002/09/01-2006/08/31

DESCRIPTION (provided by applicant): Antibiotic resistance among bacteria is a major problem in medicine. There have been repeated calls for the prudent use of antibiotics, but little is known about optimizing use to conserve efficacy. A better understanding of the relationship between dosing and the selection of resistance mechanisms may be useful. We have taken an approach that integrates pharmacokinetic, bacteriological, and molecular data into a pharmacodynamic model that examines the emergence of resistance when *Staphylococcus aureus* is exposed to ciprofloxacin in an in vitro system. This system allows accurate simulations of human pharmacokinetics and monitoring of the pharmacodynamic effect on bacteria. We found that antibiotic "sensitive" (S) cultures often harbor subpopulations with low-level resistance (RL); regimens providing low antibiotic concentrations may kill S, but allow RL to survive without evolving into bacteria with high-level resistance (RH); regimens producing moderate concentrations may eradicate S, but cause RL to evolve into RH through a variety of mechanisms; and regimens producing high concentrations may eradicate S and RL strains before they evolve into RH. Thus, the evolution of RL to RH, and ultimately treatment success or failure, appears to be dependent, in part, upon antibiotic dosing. A preliminary pharmacodynamic model described the experimental data well. Based on these findings, we hypothesize that novel regimens may prevent the emergence of resistance, and these regimens can be rationally designed by understanding the effect of antibiotic concentrations on the selection of antibiotic resistance mechanisms. To test this hypothesis, we will expose bacteria to constant and fluctuating ciprofloxacin concentrations in the in vitro system and monitor the incidence and prevalence of bacteria with up-regulated efflux and/or mutations in the quinolone resistance determining regions of topoisomerase genes with conventional assays and real-time PCR. Correlations between pharmacokinetic parameters and resistance mechanisms will be used to develop alternative pharmacodynamic models that more accurately characterize the relationship between dosing and resistance. The ability of the pharmacodynamic models to predict the outcome of regimens designed to prevent (or allow) the emergence of resistance will be tested using artificially constructed cultures comprised of varying proportions of S, RL, and RH bacteria. We believe understanding the mechanisms underlying resistance will enhance our ability to design alternative dosing strategies to effect clinical cure.

<b>Grant:</b>	1R01GM066140-01	
<b>Program Director:</b>	CHIN, JEAN	
<b>Principal Investigator:</b>	KUEHN, META J	PHD OTHER AREAS
<b>Title:</b>	Production and function of E coli vesicles	
<b>Institution:</b>	DUKE UNIVERSITY	DURHAM, NC
<b>Project Period:</b>	2002/09/01-2004/08/31	

DESCRIPTION (provided by applicant): Escherichia coli, as well as all of its gram-negative relatives studied to date, undergo a process of vesiculation, or pinching-off, of the outer membrane. Vesicles have been shown to be capable of fusion with both bacterial and eukaryotic membranes, delivering soluble and membrane components during this process. Although their presence has been recognized for decades, bacterial outer membrane vesicles have yet to be investigated at a basic genetic and biochemical level. In preliminary work, methods have been developed to isolate and purify vesicles from E. coli. The first objective is a genetic approach to elucidate the cellular machinery that produces vesicles. Randomly generated E. coli mutants will be screened for defects in vesicle production. The second objective is to how vesicles benefit the "mother cell" under normal physiological conditions and under stressful growth conditions. The ability of vesicles to communicate between cells may help in a competitive growth environment and to disseminate genetic information. Using biochemical assays, we will investigate vesicle-mediated transmission of proteins, lipids and nucleic acids. Artificial liposomes have been shown to fuse with bacterial membranes in vitro and these experiments provide a basis to begin studying vesicle membrane characteristics that may be important for fusion. Under stressful growth conditions, the vesicle pathway may be co-opted to allow quick remodeling of the outer membrane. The contribution of vesicles to the dramatic switch in lipopolysaccharide composition after cold shock will be analyzed. Further, vesiculation mutants will be used to investigate the function of vesicles during a bacterial response to diverse environmental stresses. The aims of the proposal are distinct and do not depend on one another, yet build on each other to address the project hypotheses. This powerful combination of genetics and biochemistry will set a foundation for future discoveries in this basic area of bacterial physiology. It is anticipated that these studies will reveal general principles of membrane dynamics, while also highlighting new concepts unique to bacterial membranes.

**Grant:** 1R01GM066145-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** UNGER, VINZENZ M PHD  
**Title:** Role of G protein Coupling in Fe(II)-Uptake in Bacteria  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2002/09/01-2007/08/31

DESCRIPTION (provided by applicant): Iron is essential for cell function causing competition for it between pathogens and their hosts. In the gut and stomach, pathogens like *Helicobacter*, *Salmonella* and *Campylobacter* rely on the uptake of Fe(II). Although Fe(II)-uptake is critical for virulence, little is known about the mechanisms of its uptake. The goal of this project is to understand the function of the membrane protein FeoB which is essential for Fe(II)-uptake in bacteria. FeoB is both novel and unique. Notably, the amino acid sequence of FeoB predicts a GTP-binding domain that is connected to a bundle of several putative transmembrane alpha-helices. Based on this design, we hypothesize that FeoB may have served as primordial ancestor for G protein-coupled receptors and/or channels. We will employ biochemical, genetic and biophysical tools to test this hypothesis and to establish the role of FeoB for Fe(II) uptake. The results of our work are important for understanding iron homeostasis in pathogens and may enable us to identify new strategies for treating microbial infections. The first aim is to determine the function of FeoB in Fe(II) uptake. We show that the N-terminal domain of FeoB acts as a regulatory GTP alpha-binding protein. However, the function of the membrane embedded domain remains unknown. We will combine in vivo Fe(II) uptake experiments with in vitro measurements of FeoB's Fe(II)-binding and Fe(II)-transport properties to establish whether FeoB functions as transporter/channel or acts as a receptor protein. The second aim is to determine the function of FeoB's G protein in Fe(II)-uptake, and how the activity of the G protein is regulated. We will determine whether FeoB itself rather than a downstream target is regulated by the N-terminal domain and whether Fe(II) can modify the activity of the G protein. The third aim is to identify the molecular basis for a guanine-nucleotide-exchange-factor like activity that we discovered in FeoB's N-terminal domain. We will disable this activity by mutagenesis and determine the importance of nucleotide exchange for Fe(II) uptake in vivo. The fourth aim is to generate crystals of FeoB embedded in a lipid bilayer. Ultimately, this will allow visualization of FeoB, and reveal whether its structure is related to other G protein-coupled membrane proteins.

**Grant:** 1R01GM066174-01  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** KAHNE, DANIEL E PHD ORGANIC CHEMISTRY  
**Title:** Studies Toward Hybrid Glycopeptide Antibiotics  
**Institution:** PRINCETON UNIVERSITY PRINCETON, NJ  
**Project Period:** 2002/07/01-2006/06/30

Glycolipid derivatives of vancomycin are active against vancomycin-resistant bacterial strains, and thus provide a starting point for the design of better antibiotics. Using a combination of chemical, biochemical, and genetic approaches, this laboratory has obtained evidence that these vancomycin derivatives have two different modes of action. One mode of action is shared with the parent compound, vancomycin, and involves binding to the peptide termini of peptidoglycan precursors, sterically blocking the enzymes involved in peptidoglycan synthesis. The second mode of action, attributable to the functionalized carbohydrate portion of the molecules, also involves inhibition of peptidoglycan synthesizing enzymes, but does not depend on peptide binding. Instead, the functionalized carbohydrates directly inhibit bacterial transglycosylases. This latter mode of action may explain the activity against resistant bacterial strains. If so, one might predict that better antibiotics can be made by coupling a compound having transglycosylase inhibitory activity to a compound having peptide binding activity. The first aim of this grant is to investigate the requirements for structural activity of the best transglycosylase inhibitor currently known, the pentasaccharide moenomycin A. The second aim is to explore the potential of hybrid antibiotics consisting of a peptide binding element coupled to a transglycosylase inhibitory element. The third aim involves developing a better understanding of the regulatory networks involved in transglycosylation by probing bacterial strains with the compounds that are produced. Since transglycosylation is important for both growth and division of bacterial cells, understanding these networks may shed light on fundamental aspects of bacterial metabolism.

**Grant:** 1R01GM066189-01  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** HASKO, GYORGY MD  
**Title:** Adenosine in trauma and sepsis  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2002/07/01-2006/06/30

Multiple organ failure (MOF) is the cause of 50 percent to 80 percent of all deaths in surgical intensive care units. MOF is documented to occur after a number of diverse clinical conditions, including mechanical and thermal trauma, pancreatitis and shock. In a large subgroup of patients, secondary infections serve to trigger the development of MOF, which is related to the development of an excessive compensatory anti-inflammatory reaction (CARS) and a generalized immunosuppressive state. CARS is characterized by several changes in the patients' immune phenotype. Two of the most important of these immune-phenotypic changes are a shift in T helper (Th) cell population from a Th1 to a Th2 response and shift in the macrophage phenotype from a proinflammatory to an anti-inflammatory one. This shift in the macrophage phenotype is characterized by a decrease in the production of IL-12 and an increase in the production of IL-10. Neither the signals nor mechanisms responsible for the development of this altered immune phenotype have been fully elucidated. Recently, it has been proposed that this immunosuppressed state may be secondary to the excessive release of a variety of mediators including catecholamines and glucocorticoids by activation of the stress system. In addition, it appears that adenosine (ADO), another stress mediator released excessively during CARS, could also contribute to the immune paralysis seen in CARS, since ADO appears to potentiate the development of an immune compromised state. Using an anti-CD3- stimulated mouse spleen cell system, we have recently discovered that extracellular ADO augments the production of the Th2 cytokine IL-4, whereas it reduces the production of the Th1 cytokine interferon-gamma. In addition, we have obtained evidence that ADO enhances IL-10 and decreases IL-12 production by immunostimulated macrophages. Thus, we will investigate the hypothesis that high extracellular concentrations of ADO may contribute to the deleterious immune hyporesponsiveness observed in patients with CARS. Because ADO exerts its biological effects by binding to any of 4 specific cell surface receptors, we also hypothesize that ADO shifts the immune response from a proinflammatory to an anti-inflammatory one through the activation of certain ADO receptors present on T cells and macrophages. We will test these hypotheses both in vitro using T cell and macrophage systems as well as in vivo using the mouse cecal ligation and puncture model of MOF.



**Grant:** 1R01GM066202-01  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** COOPERSMITH, CRAIG M MD  
**Title:** Gut Epithelial Apoptosis in Shock and Sepsis  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2002/07/01-2006/06/30

Sepsis and noninfectious inflammation lead to organ dysfunction and death in greater than 230,000 people annually in the United States alone. Recent studies demonstrate that sepsis and noninfectious inflammation induce excess intestinal epithelial apoptosis in both animal models of critical illness and in human autopsy studies. The central hypothesis of this new laboratory is that increased gut epithelial apoptosis is detrimental in critical illness, and that decreasing levels of cell death will improve experimental survival. Since previous descriptive studies showing elevated intestinal epithelial apoptosis in sepsis and noninfectious inflammation cannot distinguish whether altered gut apoptosis has a functional significance in critical illness, the first aim of this investigation is to demonstrate that inhibiting gut epithelial apoptosis through mechanistically distinct strategies decreases mortality in diverse models of critical illness. Gut epithelial apoptosis will be selectively targeted using transgenic mice that overexpress Bcl-2 in their intestinal epithelium. Gut-directed caspase inhibitors will also be utilized. These apoptosis-inhibition strategies will be complemented with a novel apoptosis-acceleration strategy using the antilymphocyte antibody, anti-CD3 which causes a 40-fold induction of gut epithelial apoptosis. Mechanisms that may underlie the survival advantage conferred by a decrease in gut apoptosis will be investigated as well. Gut permeability studies will be performed using the ex vivo everted gut sac model on transgenic animals that overexpress intestinal Bcl-2 and their control littermates after induction of sepsis or noninfectious inflammation and. Interactions with the immune system will be assessed by the development of mice that simultaneously overexpress Bcl-2 in their intestinal epithelium but are deficient for T- and B- lymphocytes (Rag-1 mice). Throughout these studies, four models of critical illness will be utilized varying the site of injury (intraabdominal vs. lung) and type of infection (polymicrobial vs. monomicrobial).

**Grant:** 1R01GM066269-01  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** TAN, MAN-WAH MA  
**Title:** Genome-wide Dissection of *c. elegans* Innate Immunity  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 2002/08/01-2007/07/31

DESCRIPTION (provided by applicant): Our goal is to understand the mechanisms of innate immunity at the molecular level. The innate immune system provides the body with its first line of defense against infections and is crucial for survival. Many human diseases result from a failure of the innate immune system. In order to identify and characterize novel mechanisms and effectors of the innate immune system, we will use the infections of *C. elegans* by several human bacterial pathogens - *Pseudomonas aeruginosa*, *Salmonella enterica* and *Enterococcus faecalis* - as a model. *C. elegans* is an excellent model for the study of innate immunity; it allows us to combine the power of genetic and functional genomic approaches to systematically and comprehensively dissect the innate immune system. For this proposal, we seek to address the following questions. Within a single organism, what are the molecules that make up the innate immune system? What intracellular pathways are triggered in response to infections by different classes of bacterial pathogens? What molecules are produced that directly destroy or inhibit the growth of the invading pathogens? We will use a variety of approaches, including the combination of genetic screens, full genome gene expression profiling, bioinformatic searches for homologous sequences known to be involved in the innate immune response, and epigenetic inhibition of gene function by double-stranded RNA interference (RNAi) to address the above questions. *C. elegans* has an inducible defense system and uses the evolutionarily conserved MAP kinase and TGF-beta pathways for defense against bacterial infection. The MAP kinase and TGF-beta pathways have also been implicated in innate immune response in *Drosophila* and in mice, respectively. Thus, we also propose to identify downstream targets to the TGF-beta pathway, and to determine how the TGF-beta pathway interacts with the MAP kinase pathway in mediating antibacterial defense. Because the signaling pathways in anti-bacterial defense are conserved across phylogeny, these studies should provide significant insights into anti-bacterial response in other organisms, including humans.

**Grant:** 1R01GM066303-01  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** BRENT, TAMARA L MD  
**Title:** Synthesis & transport of cryptococcal capsule precursors  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2002/09/01-2006/08/31

DESCRIPTION (provided by applicant): *Cryptococcus neoformans* is an opportunistic fungal pathogen, distinguished by a complex polysaccharide capsule required for virulence. The broad, long-term objectives of this work are to better understand the upstream metabolic events required for capsule synthesis in *C. neoformans*. The specific aims are directed at understanding the steps that provide the nucleotide sugar precursors essential for construction of the two major capsular polysaccharides, glucuronoxylomannan (GXM) and galactoxylomannan (GaIXM). These upstream steps are crucial for successful capsule construction, and therefore for cryptococcal virulence. Aim I focuses on a UDP-glucose dehydrogenase responsible for generating UDP-glucuronic acid, the activated donor of the glucuronic acid which is a major component of GXM. This protein has been expressed in active form, and efforts will focus on biochemical characterization, inhibitor studies, and gene disruption. Mutants defective in the activity will be generated and studied for their phenotype, virulence, and capsule structure. Aim II concerns a cryptococcal UDP-glucuronic acid decarboxylase discovered by the investigator and her coworkers, which is the first enzyme of its class to be cloned and expressed. This enzyme converts UDP-glucuronic acid to UDP-xylose, the donor of the xylose that is present in both major capsule glycans. *C. neoformans* cells disrupted in this gene demonstrate altered capsule structure and dramatically reduced virulence. Experiments will address the active site of the enzyme, enzyme inhibitors, and three dimensional structure. Aim III addresses the transport of nucleotide sugars into compartments in the cell where they serve as substrates for glycan synthesis. Initial studies will focus on a transporter cloned by the investigator's group. This protein will be characterized by in vitro assays and in vivo experiments, and a mutant with reduced expression of the protein will be tested for virulence and biochemically characterized. Other proteins identified by sequence analysis as putative transporters will be investigated, both by using RNA interference, recently shown by the investigator to effectively down-regulate gene expression in *cryptococcus*, and by biochemical studies. All of the processes under study are required for capsule synthesis, and are therefore essential for virulence of *C. neoformans*. Advances in understanding the biochemistry of nucleotide sugar synthesis and transport in this pathogen, and in particular progress in identifying inhibitors of the proteins involved, should contribute to the development of effective strategies for antifungal therapy.

**Grant:** 1R01GM066356-01  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** YU, XIAO-QIANG PHD  
**Title:** Lectins in Insect Immunity  
**Institution:** KANSAS STATE UNIVERSITY MANHATTAN, KS  
**Project Period:** 2002/04/01-2002/08/31

DESCRIPTION: (provided by the applicant): Non-self recognition is an essential component of the insect defense system to fight infection. Recognition of non-self in insects is mainly accomplished by a set of pattern recognition receptors, which are proteins that bind to polysaccharides in the surface of pathogens or parasites. Such recognition initiates a variety of immune responses, including prophenoloxidase (PPO) activation and encapsulation. PPO activation involves a serine proteinase cascade, leading to melanotic encapsulation of metazoan and protozoan parasites. In insect vectors of human diseases, detection and killing of parasites are not highly effective, perhaps because certain recognition receptors are lacking or do not interact with other molecules to stimulate an adequate protective response. Little is known about the recognition process mediated by any pattern recognition receptors in insects, or the mechanisms by which a variety of immune responses are initiated by the recognition process. Lectins are primary candidates as pattern recognition receptors because they can bind to terminal sugars of glycoproteins and glycolipids on the surface of many pathogens. Four novel C-type lectins, immulectins (IMLs), have been isolated from the tobacco hornworm, *Manduca sexta*. IMLs are involved in PPO activation and encapsulation. Also, two IML-2-associated serine proteinase homologs (SPHs) identified in *M. sexta* are necessary for PPO activation by prophenoloxidase-activating proteinase (PAP). This proposal aims to test a general hypothesis that binding of IML-2 to the surface of a pathogen or parasite functions to localize phenoloxidase (PO) activation to the area surrounding the invading organism. A part of this hypothesis is that IML-2 bound to a parasite surface participates in assembly of a complex of plasma proteins that results in activation of PPO specifically at the surface of the invader. The specific aims are: 1. Investigate binding specificity of IML-2. The binding of IML-2 to carbohydrates on the surface of pathogens or parasites triggers immune responses. Further research is needed to determine the carbohydrate binding specificity of IML-2. 2. Study IML-2-SPH initiated assembly of a protein complex at a surface during PPC activation. Formation of hemolymph protein complexes on the surface of parasites may localize PPO activation on the site of invasion or on the surface of invading parasites. Experiments will be designed to investigate protein-protein interactions among IML-2, SPH, PPO, and PAP.

**Grant:** 1R01GM066466-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** YEH, JOANNE I BS  
**Title:** Structural Studies of Metabolic Membrane Proteins  
**Institution:** BROWN UNIVERSITY PROVIDENCE, RI  
**Project Period:** 2002/08/01-2007/07/31

DESCRIPTION (provided by applicant): We have obtained diffracting crystals of two membrane proteins involved in the glycerol metabolic pathway in bacteria, glycerol facilitator and glycerol-3-phosphate dehydrogenase. This is a significant step towards crystal structure determination and these structures will yield valuable new insight, linking protein folds to function and protein-protein interactions as well as mechanisms of catalysis, regulation, and transmembrane uptake of solute molecules. Underlying effective mechanisms of bacterial proliferation are means of mediating oxidative and carbohydrate metabolism. This structural study focuses on elucidating structure-function relationships of key bacterial membrane proteins that mediate fundamental oxidative and carbohydrate metabolism. We have been engaged in structural studies of these metabolic proteins for several years and are at pivotal point in integrating all of our results into a comprehensive and coherent picture of metabolism in these pathogenic bacteria. We have determined the structures of three other members of the oxidative and glycerol metabolism pathways, including NADH peroxidase, NADH oxidase, and glycerol kinase. This proposal addresses key questions correlating structure to function and regulation. Gly-3-phosphate dehydrogenase is of particular medical importance as it is a key player in providing triose phosphate intermediates for biosynthesis of polysaccharides that form biofilm and protects the bacterium from dehydration as well as antibiotic therapy. This membrane-protein structural study has the potential to yield new and novel results, which are not provided by high-throughput structural genomics. We have overcome some of the most difficult and rate-limiting hurdles in membrane protein structural studies by obtained diffracting crystals of the glycerol facilitator (2.4 Å) from Gram-positive *Streptococcus pneumoniae*, a membrane protein involved in glycerol uptake and likely to be regulated via protein-protein interactions with glycerol kinase, whose structure we've recently determined. Subtle but significant differences exist between this and the Gram-negative *E. coli* facilitator. We have diffracting crystals of the glycerol-3-phosphate dehydrogenase (3.3 Å) from *Pseudomonas aeruginosa* and have promising indications for better diffracting crystals. Our specific aims are to obtain atomic resolution structures of both membrane proteins, both of which are likely to be novel targets for antibiotic design and therapy.

**Grant:** 1R01GM067245-01  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** STEWART, PHILIP S PHD  
**Title:** Antibiotic Susceptibility of Bacteria in Biofilms  
**Institution:** MONTANA STATE UNIVERSITY (BOZEMAN) BOZEMAN, MT  
**Project Period:** 2002/08/01-2006/07/31

DESCRIPTION (provided by applicant): When bacteria attach to a surface and grow as a biofilm they are protected from killing by antibiotics. Biofilm formation is increasingly recognized as a factor in the persistence of varied infections. The goal of this project is to complement ongoing experimental investigations of antibiotic resistance in biofilms by developing the first comprehensive, phenomenological model of biofilm reduced susceptibility to killing by antibiotics. An existing mathematical model of biofilm development will be expanded to include four hypothesized protective mechanisms. These mechanisms address retarded antibiotic penetration, reduced metabolic activity or growth in parts of the biofilm due to local nutrient depletion, stress response activation by some biofilm bacteria, and differentiation of some biofilm cells into a dormant persister state analogous to spore formation. The model will be improved by developing mathematical expressions for the release of cells from the biofilm based on a mechanical analysis of the biofilm as a viscoelastic fluid. Finally, model results will be compared to experimental data. Experiments will be performed to measure spatio-temporal responses, including both killing and detachment, to antibiotic treatment in a *P. aeruginosa* experimental system, and these results will be compared with output of the mathematical model. Progress in understanding the stubborn persistence of biofilm infections in the face of antibiotic chemotherapy has been surprisingly slow. This modeling effort will accelerate this effort by integrating the many constituent phenomena that must be considered and serving as a vehicle for dialogue between the diverse disciplines that must communicate to solve this problem. The model will ultimately be a tool for investigating the consequences of hypothesized resistance mechanisms, designing experiments to test these mechanisms, identifying novel treatment strategies, and determining optimal antibiotic dosing protocols. This project will afford a rich interdisciplinary training experience for the three participating graduate students.

**Grant:** 1R01GM067248-01  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** CHOPP, DAVID L PHD  
**Title:** Cell-To-Cell Signaling in Bacterial Biofilms  
**Institution:** NORTHWESTERN UNIVERSITY EVANSTON, IL  
**Project Period:** 2002/07/01-2007/06/30

DESCRIPTION (provided by applicant): The tendency of bacteria to stick to surfaces and form surface-associated communities called biofilms has been well documented. A hallmark characteristic of biofilms is that they can be up to a thousand times more resistant to antimicrobial stress than free-swimming cells of the same species. *Pseudomonas aeruginosa* is an opportunistic human pathogen that has been implicated in nosocomial infections as well as chronic lung infections in people suffering from Cystic Fibrosis. *P. aeruginosa* uses a cell-to-cell signaling mechanism called quorum sensing to regulate virulence factor production, as well as biofilm formation. Therapeutic strategies directed at quorum sensing may be effective at combating *P. aeruginosa* biofilm infections. The proposed research will develop and utilize mathematical models for predicting acyl-HSL-regulated gene expression in a biofilm system. These models will be tested experimentally against actual biofilms grown under ecologically relevant conditions. The ultimate goal is to generate a versatile, predictive model that will allow clinicians to predict the onset of quorum sensing-regulated gene expression during the course of biofilm infections. A hierarchy of mathematical models will be developed for three different length scales: single unit cell, small clusters of cells, and full biofilm. The objective of these models will be to predict the level of acyl-HSL within an experimentally observed biofilm and its relationship to its environment. New hybrid numerical methods will be employed to simulate the mathematical models which will be based upon a coupling of the Level Set Method and the Extended Finite Element Method. The new method will be uniquely suited for simulating the growth of the biofilm and the synthesis of the signal acyl-HSL. Experimental data for parameter estimation in the models, as well as model testing will be done using a battery of biological reporter systems. These reporter strains utilize transcriptional fusions of quorum sensing-regulated promoters to the green fluorescent protein (GFP). The expression of GFP and the onset of fluorescence coincide with the accumulation of acyl-HSL signal to a critical threshold concentration. Use of these reporter systems in conjunction with scanning confocal laser microscopy will allow the examination of gene expression at the single cell level. Experimental results will be used to refine the mathematical models.

**Grant:** 2R15GM051006-03  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** COLLINS, MARY L PHD  
**Title:** Assembly of membrane protein complexes  
**Institution:** UNIVERSITY OF WISCONSIN MILWAUKEE MILWAUKEE, WI  
**Project Period:** 1997/06/01-2005/08/31

DESCRIPTION (provided by applicant): An understanding of the process of membrane assembly is critical to many areas of biomedical research. Bacteria are useful model systems for the study of cellular phenomena. The photosynthetic bacterium *Rhodospirillum rubrum* forms a differentiated photosynthetic membrane under certain growth conditions and provides an excellent opportunity to study the process of membrane assembly. *R. rubrum* is an experimentally accessible system because: 1) the photosynthetic membrane has a simple protein composition consisting mainly of the photochemical components; 2) the formation of the photochemical complexes and the assembly of the photosynthetic membrane can be experimentally manipulated; 3) functional assembly of these components can be assessed on the basis of spectroscopy and the capacity for phototrophic growth; 4) membranes are readily isolated from this organism; 5) nonphotosynthetic mutants are viable because they can grow chemotrophically; and 6) the photochemical components are among the most well studied of membrane protein structures. Our long-term goal is to examine the events in the assembly of membrane protein complexes using the photochemical components of *R. rubrum* as a model system. In this work, the assembly of the photochemical components and the role of putative proteins encoded within the photosynthetic gene cluster will be investigated by the construction, complementation and characterization of mutants. These studies will help to define the mechanism and requirements for the assembly of these membrane-protein complexes. This work is based on the results of previous studies and enabled by the expression vector we have developed. This work will lead to an enhanced understanding of the process of membrane protein assembly.



**Grant:** 2R15GM057636-02  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** GAVINI, NARA PHD  
**Title:** Docking Site Mutations in the Fe-and MoFe-proteins.  
**Institution:** BOWLING GREEN STATE UNIV BOWLING BOWLING GREEN, OH  
GREEN  
**Project Period:** 1998/05/01-2006/03/31

The nitrogenase is comprised of two separately purified proteins, the Fe- protein and the MoFe-protein. Interactions between the Fe-protein and the MoFe-protein occur at many levels; during the maturation and assembly of nitrogenase and during the biological nitrogen fixation reaction. Both proteins acquire different conformations during these interactions. The specific aim of this research proposal is to elucidate the regions of the Fe-protein and the MoFe-protein that play important roles in the different conformations these proteins acquire for their interactions during the maturation and assembly of the nitrogenase and also during the biological nitrogen fixation reaction. Previously we have shown that the *A. vinelandii* UW97 is Nif<sup>+</sup> due to a mutation, Ser44Phe, in the Fe- protein component. Utilizing this strain, we have isolated compensating suppressor mutations by using a genetic approach. The genetic approach was directed to isolate spontaneous Nif<sup>+</sup> revertants. Characterization of two of these revertants showed that both of them have retained the original SerPhe mutation and carried second site compensating suppressor mutations in either the Fe-protein or in the  $\alpha$  and  $\beta$  subunit of the MoFe-protein. During this grant period we proposed purify these mutated Fe-proteins and the mutated MoFe-protein and characterize their biochemical properties, and ability to interact with each other or with their wild type counterparts. We have also isolated targeted compensating mutations either in *nifH*, or *nifK* genes. This method involves propagating the cloned genes in the *E. coli* strain XL1-RED to introduce random mutations in these genes. By using this strategy, to date we have isolated several clones of *A. vinelandii* UW97 revertants. These clones were subjected to genetic analysis and confirmed that the compensating mutations of these suppressor mutants are located in the DNA fragments encoding *nifH*, *nifD* or *nifK*. Here we propose to identify exact locations of these compensating mutations by involving undergraduate students in this research, purify the altered nitrogenase from these strains, and characterize the functional properties by involving graduate students. The advantages of the approaches we have taken here is that they provide opportunity to unravel new regions of the Fe-protein and the MoFe-protein that are important for their interactions during maturation of the Fe-protein and biological nitrogen fixation reaction. We believe that these studies will provide new insights regarding how the components of this complex metalloenzyme maintain such high specificity in their interactions.

**Grant:** 2R15GM057779-02  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** CHAMPNEY, WILLIAM S AB  
**Title:** Antibiotic Inhibition of Bacterial Ribosome Formation  
**Institution:** EAST TENNESSEE STATE UNIVERSITY JOHNSON CITY, TN  
**Project Period:** 1998/07/01-2005/02/28

DESCRIPTION (provided by applicant): The current resurgence of antibiotic-resistant organisms underscores the importance of gaining a better understanding of antibiotic mechanisms, resistance modes and the structural features necessary for optimal effectiveness. The overall objective of this proposal is to learn how five structurally different antibiotics inhibit the process of bacterial cell growth. This investigation will explore the new observation that macrolide antibiotics as well as the ketolides, lincosamides, streptogramin B compounds and oxazolidinones can all inhibit the assembly of the large ribosomal subunit in bacterial cells. Ribosome formation will be analyzed in *Staphylococcus aureus* and *Escherichia coli* cells to define the inhibitory features of these compounds. The mechanism of subunit assembly inhibition will be tested by examining the components of the subunit precursor particles which accumulate in the presence of the antibiotic. Aspects of the breakdown of the inhibited assembly intermediate will also be studied. Ribosomal subunits will be reconstituted from component RNAs and proteins to define the molecules involved as targets for assembly inhibition. An investigation of this assembly-sensitive site and the mode of inhibition of assembly will reveal how certain antibiotics can have two inhibitory activities. The findings from this work will help in assessing the effectiveness of existing antibiotics and in developing new compounds as antimicrobial agents.

**Grant:** 1R15GM064406-01  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** MEHL, ANDREW F PHD  
**Title:** Studying GrpE: Protein Function, Stability, and Folding  
**Institution:** KNOX COLLEGE GALESBURG, IL  
**Project Period:** 2002/06/01-2006/05/31

DESCRIPTION (provided by applicant): The GrpE protein from E. coli is an essential component of the heat shock protein 70 (Hsp70) molecular chaperone machine which also includes the DnaJ and DnaK proteins. These proteins work together to help facilitate proper protein folding by preventing proteins from going down unwanted pathways such as protein aggregation. They are also involved in the transport of proteins across membranes and the assembly and disassembly of large protein and or protein/DNA complexes. GrpE has some very interesting and unique features concerning its homodimeric structure. There is a long "tail" region at the NH2-terminal end that is composed of two alpha-helices paired together (one from each monomer), and also there is an extended polypeptide from each monomer at the very end that are not paired. The dimer interface includes the "tail" and a four-helix bundle region where each monomer contributes two short ahelices. There is also a beta-sheet domain at the COOH-terminal end that is not involved in the dimer interface. The interaction between GrpE and DnaK takes place between only one of the two monomers in the dimer structure of GrpE and mainly with the beta-sheet domain. The goal of the proposed research is to learn more about the structure of the GrpE protein with emphasis on these unique structural features and interaction with DnaK. Additionally, there are goals that are aimed at investigating mechanisms and rules that govern protein oligomerization and folding. Insight into the mechanisms for the formation of a protein with a four-helix bundle at the dimer interface (GrpE) will help contribute the general knowledge about protein structure and folding. Experimentally, a mutational approach will be taken. Specific sequence deletion mutants of GrpE that contain certain regions of the protein will be created and then tested for functions, such as dimerization, interaction with DnaK. Point mutants will also be created to test the function of salt bridges within the dimer interface. An internal deletion mutant is proposed that will potentially lead to only a monomer of GrpE.

<b>Grant:</b>	1R15GM064445-01	
<b>Program Director:</b>	ANDERSON, JAMES J.	
<b>Principal Investigator:</b>	ALLEN, MARY M	PHD
<b>Title:</b>	The effects of acidic pH on cyanobacteria	
<b>Institution:</b>	WELLESLEY COLLEGE	WELLESLEY, MA
<b>Project Period:</b>	2002/03/01-2006/02/28	

DESCRIPTION (provided by applicant): The objective of the proposed research is to study the effect of acidic stress on cyanobacteria. The long term objectives of this laboratory have been to relate cyanobacterial growth and physiology to environmental variables and to determine the mechanisms by which cyanobacteria regulate the expression of their genes under stressful conditions. The hypotheses to be tested in the proposed research are that cyanobacteria have reproducible and specific responses to acidic stress, that they display an acid tolerance response to changes in external pH that allows the regulation of homeostasis, and that specific proteins are induced in response to acidic stress. <sup>31</sup>P NMR spectroscopy will be used to determine internal pH of cells in various medium pHs and cell densities. <sup>23</sup>Na NMR spectroscopy, using aqueous shift reagents, will allow the measurement of intracellular sodium concentrations in order to determine if a cation/proton antiporter is involved. Viability will be assessed using fluorescent microscopy and image analysis. One- and two-dimensional gel electrophoresis and autoradiography will be carried out to determine the various proteins that are induced during acid tolerance and stress. Granules that appear when cells are acid stressed will be studied and identified by confocal microscopy, NMR spectroscopy and chemical assay. How bacteria survive in stressful environments is an intriguing biological problem that will lead to understanding the basic cellular biology and physiology of both pathogenic and environmentally important microorganisms. New functions for cellular proteins may be described from the proteomic studies.

**Grant:** 1R15GM064447-01A1  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** VARY, PATRICIA S  
**Title:** Sequencing of the Largest Plasmids of *B. megaterium*  
**Institution:** NORTHERN ILLINOIS UNIVERSITY DEKALB, IL  
**Project Period:** 2002/07/01-2005/06/30

DESCRIPTION (provided by applicant): *B. megaterium* strain QM B 1551 harbors a multiplasmid array of seven plasmids, 11% of the total cellular DNA. The long-term goal of this study is to thoroughly investigate one multiplasmid "genome" to provide much needed information on plasmid biology, the role of plasmids in the cell, plasmid transfer and distribution among the Gram-positive bacteria, and the potential role in the emergence of new pathogens. Three of the smallest plasmids (5.4, 9.1 and 26kb) have been completely sequenced and a fourth (55 kb) is 70% sequenced. Six replicons have been characterized, and genes potentially of use in bioremediation, transfer and vector construction, as well as a complete rRNA operon have been found. This proposal is a continuation of a grant in which the replicon of the third largest plasmid, pBM500 (71 kb) has been characterized, flanking DNA cloned, the source plasmid identified, and a study initiated to test host range. A pilot study of a pathogenic *B. megaterium* isolate (the first ever reported) has led to the discovery that genes cross-hybridizing with *B. cereus* virulence genes are present on the chromosome of the isolate and strain QM B 1551. This proposal is for a considerable expansion of the first grant in which the remaining plasmid DNA (400 kb) will be shotgun-cloned and sequenced in collaboration with Integrated Genomics. Annotation and initial functional genomics will be done using powerful new programs at both NIU and IG. This is the first attempt to completely sequence and characterize a multiplasmid array and should reveal many interesting plasmid genes for further annotation and functional analysis. From results with the smaller plasmids, it is expected that genes for transfer, unusual metabolic enzymes, and other unknown but significant processes will be found. To continue testing for host range and horizontal transfer, the replicons will be used in hybridizations, conjugations, and transformations to other *Bacillus* and other Gram positive genera to determine the host range, distribution, and transfer capabilities of the plasmids. The information obtained should substantially increase the database of plasmid genes and lead to a more comprehensive understanding of the role of these plasmids in the cell and of their potential applications in medicine, bioremediation and vector construction.

**Grant:** 1R15GM064511-01  
**Program Director:** JONES, WARREN  
**Principal Investigator:** MEGANATHAN, RANGASWAMY PHD  
**Title:** Studies on Vitamin K Biosynthesis  
**Institution:** NORTHERN ILLINOIS UNIVERSITY DEKALB, IL  
**Project Period:** 2002/03/01-2006/02/28

DESCRIPTION (provided by applicant): Humans and animals cannot synthesize vitamin K (K). K dependent, hypoprothrombemia is an important clinical problem under a number of conditions. The K requirement in human and animal nutrition is met by green plants and intestinal bacteria. Because K plays an important role in human well being, it is important to understand its biosynthesis and regulation. The vitamin K biosynthetic pathway may be summarized as follows: chorismate --menF--> isochorismate --menD--> 2-succinyl-6-hydroxy-2, 4-cyclohexadiene-1 - carboxylate (SHCHC) ---menC----> o-succinylbenzoic acid (OSB)-menE-> o-succinylbenzoyl-CoA (OSB-CoA) --menB-->{ 1, 4-dihydroxy-2-naphthoyl-CoA (DHNA-CoA)}---menH---> 1, 4-dihydroxy-2-naphthoic acid (DHNA) ---menA---> demethylmenaquinone (DMK) --- ubiE --> menaquinone (MK). Seven ORFs (six of which have been shown to be menaquinone biosynthetic genes) lie in a cluster in the order orf101, menF, menD, menH, menB, menC, and menE. Transcription of all the genes are initiated from a single strong promoter site, 26 bp 5'to orf101 was detected under anaerobic conditions. The menA gene is located at 89 min on the chromosome and is linked to an unidentified orf (orf161). In this study, we propose 1) to determine the role of orf101; 2) to study the regulation of the men operon using lacZ fusions; 3) to determine the structure of a proposed intermediate (DHNA-CoA); 4) to identify the methyltransferase involved in the DMK-->MK conversion, and 5) to characterize the active site mutants of OSB synthase, whose three dimensional structure was recently solved by us.

**Grant:** 1R15GM065121-01  
**Program Director:** CARTER, ANTHONY D.  
**Principal Investigator:** TRUN, NANCY J PHD  
**Title:** Molecular Dissection of Chromosome Folding in E coli  
**Institution:** DUQUESNE UNIVERSITY PITTSBURGH, PA  
**Project Period:** 2002/03/01-2006/02/28

DESCRIPTION: (provided by applicant): This proposal is concerned with the molecular analysis of the genes and proteins required for chromosome condensation in prokaryotic cells. The ultimate goal of the project is to thoroughly understand the molecular mechanisms of this complex, fundamental process. E. coli is used as the model system for these studies due to the extensive genetic and biochemical tools readily available for experimental use. The intent of the proposal is to study novel and critical cellular components that are required to fold the chromosome into a compact yet functional structure. The components and mechanisms identified in these studies will lead to a greater understanding of how this essential process is accomplished. Given that chromosome folding is essential for cell viability, it is likely that these studies will identify potential new targets for therapeutic agents. Using the novel genetic selection of resistance to the DNA unfolding agent camphor, a previously unknown condensing system containing three components, CrcA, CspE and CrcB, was identified. The central component of this system is CspE, a 69 amino acid, nucleic acid-binding protein. The experiments described in this proposal undertake a complete structural and functional analysis of CspE. Mutations that genetically separate the different phenotypes of cspE will be isolated using two, independent approaches. Pilot studies indicate that one of these approaches can be used successfully. The proteins produced from the wild-type gene and the mutated genes will be isolated and characterized in a series of in vitro assays. Prokaryotic cells contain several small, DNA-binding proteins that play a role in chromosome folding. All of these proteins are involved in many, different cellular processes. In this respect, CspE is yet another member of the class. What sets CspE apart from these others proteins and makes it the most amenable member of the class for the study of a direct role in chromosome folding is the ability to genetically separate its different functions by mutation. The characterization of the mutant CspE proteins described in this proposal will lead to a direct understanding of how this class of small, nucleic acid-binding proteins function in the essential process of chromosome folding.

**Grant:** 1R15GM065170-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** KREBS, MARK P PHD  
**Title:** STRUCTURAL BASIS OF BACTERIORHODOPSIN BIOGENESIS  
**Institution:** ILLINOIS STATE UNIVERSITY NORMAL, IL  
**Project Period:** 2002/04/15-2005/03/31

DESCRIPTION (provided by applicant): The long-term goal of the proposed research is understand the structural basis of membrane protein biogenesis. The focus is on the polytopic (multispanning) membrane protein, bacteriorhodopsin, an integral membrane protein produced by the archaeon *Halobacterium salinarum*. This protein folds to form a bundle of seven transmembrane alpha-helices, binds retinal and assembles in a two-dimensional crystalline lattice known as the purple membrane. Bacteriorhodopsin is a model for understanding membrane protein insertion, folding and assembly because its three-dimensional structure is known at high resolution in the native membrane environment. The objective in this proposal is to test in vivo whether bacteriorhodopsin fits a two-stage model in which interactions between transmembrane segments are the primary determinant of folding and assembly. The specific aims are (I) to introduce mutations throughout BR by a saturation mutagenesis approach implemented in preliminary studies; (II) to identify those residues at which substitutions can be tolerated without affecting the formation of BR from BO and retinal; and (III) to determine residues at which substitutions can be tolerated without affecting the assembly of the BR lattice. The results from this analysis will be compared with the three-dimensional structure of BR to test whether the predictions of the two-stage model are confirmed. These studies are expected to yield important new information on the structural basis of integral membrane protein folding and assembly in the cell.



**Grant:** 1R15GM065837-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** ENOS-BERLAGE, JODI L PHD  
**Title:** Investigating the role of calcium in bacteria  
**Institution:** LUTHER COLLEGE DECORAH, IA  
**Project Period:** 2002/07/01-2005/06/30

DESCRIPTION (provided by applicant): In order to understand bacterial pathogenesis, it is essential to have a good understanding of bacterial physiology. One area that is particularly lacking in information is the role of calcium in prokaryotes. The long-term goal of this research project is to gain an understanding of the function of calcium in bacteria. Of particular interest are regulatory functions of calcium and potential connections between calcium and bacterial pathogenesis. The marine bacterium and significant human pathogen *Vibrio parahaemolyticus* is an ideal model system for this study. This organism occupies environments that differ substantially in calcium content, for example, seawater compared to the human gastrointestinal tract. Significantly, preliminary results suggest that calcium affects a number of *V. parahaemolyticus* cellular processes, including processes that may play a role in pathogenesis. In addition, results strongly suggest that calcium regulates gene expression in this organism. Three specific aims are outlined in this proposal. Aim 1 is designed to identify gene products involved in calcium sensing and response. An existing strain containing a calcium-regulated lux fusion will be mutagenized using a transposon to identify insertions that disrupt calcium regulation. Experiments outlined in Aim 2 are designed to determine the extent of calcium-regulated gene expression in *V. parahaemolyticus* and identify regulated genes. Two-dimensional gel electrophoresis will be used to analyze protein expression in response to altered calcium levels. Mutagenesis with a transposon containing a promoterless reporter gene will allow identification of genes that are calcium-regulated. The goal of Specific Aim 3 is to thoroughly investigate the phenotypic effects of calcium in wild type *V. parahaemolyticus* and to phenotypically characterize the mutants isolated in Aims 1 and 2. Together, these experiments are expected to provide significant insight into the role of calcium in bacteria, thereby filling a gap in the current understanding of bacterial physiology. This study may increase the understanding of the role of calcium in bacterial pathogenesis. Finally, as this proposal is based primarily on a genetic approach, this project will provide ideal undergraduate research projects for students at Luther College.

**Grant:** 1R15GM065839-01  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** JAYASWAL, RADHESHYAM K  
**Title:** Role of Msr in Oxidative Stres in *S. aureus*  
**Institution:** ILLINOIS STATE UNIVERSITY NORMAL, IL  
**Project Period:** 2002/07/01-2005/06/30

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is a major cause of human pathogen that has caused a significant clinical due to acquisition of resistance to most available antibiotics. In an attempt to understand the bacterial response to cell wall-active antibiotics, nine staphylococcal proteins were identified as up-regulated employing 2-D gel electrophoresis (Singh et al., 2001). Five of these proteins were found to be homologues of an enzyme, methionine sulfoxide reductase (MsrA); signal transduction protein (TRAP); transcription elongation factor GreA; the heat shock protein GroES; and the enzyme IIA component of the PEP: sugar phosphotransferase system. We have constructed knockout mutants of the genes encoding TRAP and MsrA. Initial studies suggest that the mutations did not affect the antibiotic resistance, however, the TRAP mutant was defective in bacterial pathogenesis and the MsrA mutant showed increased sensitivity to oxidative stress. Although the role of MsrA in oxidative stress tolerance is well established, there is growing evidence suggesting the protein to be involved in multiple physiological functions. The long-term goal of this project is to study *S. aureus* MsrA for its roles in oxidative stress tolerance, antibiotic resistance and pathogenicity. The specific objectives of this study are: i) Molecular analysis of staphylococcal msrA genes: analysis of *S. aureus* genome sequence indicates the presence of a gene in the bacterial chromosome that codes for another MsrA homologue (GI: 12656489) in addition to the MsrA that we have identified to be upregulated by wall-active antibiotics. We intend to clone both the genes, overexpress them in *Escherichia coli*, and purify individual proteins to determine the associated methionine sulfoxide reductase activity; ii) Construction of msrA knockout mutants: both the msrA genes will be knocked out individually. Subsequently, a double msrA mutation will be constructed by the propagation of one mutation to the other; iii) Physiological studies: the msrA mutants will be tested for oxidative stress tolerance against various oxidative agents, such as H<sub>2</sub>O<sub>2</sub>, paraquat, AAPH. The impact of mutations on antibiotic resistance will also be determined; iv) Immunological studies: polyclonal antibodies against purified proteins will be raised to study the expression of the two MsrA homologues by Western blotting; v) MsrA in pathogenesis: the msrA mutants will be tested for their effect on bacterial pathogenicity using a rat model of endocarditis as described earlier (Mani et al., 1993); vi) Crystallographic studies: to identify the active site, we will determine the crystal structure of a protein encoded by a gene usually present downstream of many bacterial msrA genes. The results of the proposed studies are expected to provide detailed understanding of the MsrA in staphylococcal physiology and pathogenesis.

**Grant:** 1R21GM062482-01A1  
**Program Director:** ANDERSON, RICHARD A.  
**Principal Investigator:** MURPHY, KENAN C PHD  
**Title:** RECOMBINOGENIC ENGINEERING OF PATHOGENIC BACTERIA  
**Institution:** UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA  
WORCESTER  
**Project Period:** 2002/04/01-2004/03/31

DESCRIPTION (Provided by applicant): Gene deletion and/or replacement is the single most important tool for definitively identifying critical functions of infectivity and virulence in pathogenic bacteria. Yet the tools available to make such gene replacements in pathogenic bacteria have, for the most part, remained unchanged for the last 10 years. While genome sequencing projects continue to increase the number of open reading frames available for genetic analysis, gene knock-out technology in many bacterial systems remains technically cumbersome, and in some cases, unfeasible. This project is designed to explore a novel methodology for the enhancement of gene replacement in pathogenic bacteria. The Red recombination system from bacteriophage lambda, when expressed in *Escherichia coli*, generates a hyper-recombinogenic phenotype whereby gene replacement occurs at an extremely high efficiency following transformation with small (2-3 kb) linear DNA substrates. This gene replacement scheme is unique in that plasmid-chromosome co-integrants do not have to be formed (or resolved), and prior cloning of the gene of interest is not required. PCR-generated substrates with as little as 40 bp of flanking homology are substrates for efficient Red-mediated gene replacement. The recombination intermediates generated by lambda Red are channeled into the host recombination pathway. It is this "jump start" in the initiation of recombination that likely plays a key role in the generation of the hyper-rec phenotype of lambda Red-containing *E. coli*. Since most bacteria contain homologs of many of the recombination functions described in *E. coli* (e.g., *recA*, *recBCD*, *ruvAB*), Red will likely serve to generate the same hyper-rec phenotype when expressed in other (pathogenic) strains of bacteria. This proposal is a test of this hypothesis. This project is designed to generate hyper-recombinogenic strains of *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* by expression of red and phage anti-RecBCD functions in vivo from plasmids, or by replacing the chromosomal *recBCD* genes with a red-expressing operon. The system can be set up so that the hyper-rec phenotype is transient, resulting in pathogens that are altered only within the gene of interest. This project has the potential to revolutionize the methods of genetic manipulation in microorganisms, leading to faster identification of virulence genes, greater flexibility in the genetic analysis of these genes, and the speedy generation of bacterial mutants for vaccine development.

**Grant:** 1R21GM065250-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** YEAGLE, PHILIP L  
**Title:** Three dimensional structure of a 12TM membrane protein  
**Institution:** UNIVERSITY OF CONNECTICUT STORRS STORRS-MANSFIELD, CT  
**Project Period:** 2002/05/01-2004/04/30

DESCRIPTION (provided by applicant): The challenge of membrane protein structure determination remains largely unmet. Thousands of water-soluble protein structures have been solved, while, in contrast, only a handful of membrane protein structures have been solved. Yet estimates suggest that 25-40 percent of the coding regions of genomes from higher organisms code for membrane proteins. As much as 60 percent of the drug targets of the pharmaceutical industry are a family of membrane proteins called G-protein coupled receptors, yet there are no X-ray crystal structures of any of these drug targets. The long-term goal of this project is to construct a path around the current problems in membrane protein structure determination through the development of a new approach to this problem. While this new approach does not replace X-ray crystallography, it does offer structural information where crystallization suitable for diffraction studies has proven impossible. It also offers the possibility of working on proteins whose size would make them unsuitable subjects for standard NMR techniques, particularly where the effective size of the membrane protein is increased by inclusion in a detergent micelle or in a lipid bicelle. In this R21 application, we focus on a difficult challenge: the solution of the structure for a 12 TM membrane protein that has so far proven refractory to X-ray crystallography. We will use what we have learned from the solution of the structures of two 7 TM membrane proteins and determine whether the same approach can be used to solve the three dimensional structure of a much larger membrane protein, the lac permease. This structure determination of the lac permease will prove very helpful in understanding the mechanism of transport for this and related proteins. Success of this project will provide a powerful new tool for proteomics that can relatively rapidly produce useful structural information for many families of membrane proteins.

**Grant:** 2R01HD035667-04  
**Program Director:** SPONG, CATHERINE  
**Principal Investigator:** ONDERDONK, ANDREW B PHD  
**Title:** QUANTITATIVE MICROBIOLOGIC MODEL FOR PRETERM DELIVERY  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 1999/01/01-2004/12/31

DESCRIPTION (provided by applicant): Preterm delivery (PTD) is the leading cause of infant morbidity and mortality in the United States, and prevention is a primary goal for perinatal health care. Recent evidence indicates that there is a strong association between an altered vaginal microflora during pregnancy and the occurrence of PTD. However, the role of specific microorganisms in PTD is not well understood. Recent studies in this laboratory have been directed at identifying specific microbial risk factors associated with PTD. We have established a predictive statistical model for PTD, based on these microbiologic risk factors. Our studies have identified two key populations that are associated with the occurrence of PTD. It has been shown that the levels of a bacterial phospholipase, PLA2, increase in concentration between 20 and 30 weeks of gestation in women who deliver at less than 37 weeks gestation. This increase in PLA2 correlates with the presence of *Prevotella* sp. as part of the vaginal microflora. It has also been noted that the presence of both hydrogen peroxide (H2O2) producing lactobacilli and non-H2O2 producing lactobacilli simultaneously as part of the vaginal microflora is a risk factor for PTD, separate from the presence of either strain by itself. This observation suggests that combinations of strains may have a detrimental effect on pregnancy outcome. The goal of the proposed project is to prospectively collect quantitative and qualitative microbiologic data from women at high risk for PTD and those with no identifiable risk for PTD, to determine whether the statistical model is capable of predicting PTD based on microbiologic data, in preparation for a subsequent study in which women identified as at risk for PTD by this model will be treated. In addition, the actual mechanism(s) by which bacteria cause PTD will be evaluated in order to identify microbiologic targets for any interventional study. The specific aims for this proposal are to 1) refine the predictive model derived from the initial analysis of the data collected during the first three years of this study and to validate this model for predicting PID during a prospective clinical trial, 2) to improve our understanding of the role of specific bacterial species in PTD particularly *Prevotella* sp, 3) to use molecular typing methods to determine whether specific strains of lactobacilli are more common in women delivering at less than 37 weeks gestation than those delivering at 37+ weeks gestation and whether such strains are acquired during pregnancy, and 4) to determine whether interactions between strains of lactobacilli deleterious to pregnant women occur by using cultured vaginal epithelial cells in vitro exposed to combinations of lactobacilli found in vivo.

**Grant:** 1R01HD043192-01  
**Program Director:** MACKAY, H TRENT  
**Principal Investigator:** FRASER, IAN S MD  
**Title:** Control of menstrual bleeding disturbances in women  
**Institution:** SYDNEY CENTRE FOR REPRODUCTIVE ASHFIELD,  
HLTH RES  
**Project Period:** 2002/09/27-2007/06/30

DESCRIPTION (provided by applicant): This application is designed to evaluate two promising approaches to the treatment of prolonged and frequent episodes of breakthrough bleeding which sometimes accompany the use of the implantable, progestogen-only implant Implanon. These erratic episodes of bleeding can be a major reason for discontinuation of use. There is increasing evidence that continuous exposure to progestogens results in a tendency for the endometrium to release active enzymes called matrix metalloproteinases [MMPs] which can promote premature breakdown of the tissue. Inhibition of the action of these enzymes may stabilize the endometrium and improve the bleeding pattern. A commonly used tetracycline compound, Doxycycline, has strong anti-MMP action and preliminary evidence in a mouse model of menstruation suggests that it may indeed stabilize the endometrium. There is preliminary evidence that a short course of an antiprogestosterone (Mifepristone) may also stabilize the endometrium, and it is postulated that a combination of an antiprogestosterone with estrogen may be even more effective. Preliminary evidence in mice indicates that estrogen exposure of the endometrium in the absence of progesterone strongly inhibits the formation of new blood vessels and simultaneous anti-progesterone exposure will mimic this situation. Antiprogestosterones probably also have a direct effect in inhibiting angiogenesis, and the combination maybe a clinically valuable treatment. A triple combination of antiprogestosterone, estrogen and anti MMP agent may have additive effects because of the likelihood of differing actions. This study aims to explore these possibilities in large scale clinical studies, scientific study of vascular and molecular changes in endometrium and with the exploration of molecular mechanisms in mice.

**Grant:** 1R03HD041525-01  
**Program Director:** HANSON, JAMES W  
**Principal Investigator:** NEMANI, PRASADARAO V PHD  
**Title:** Role of Complement Proteins in E. coli Meningitis  
**Institution:** CHILDREN'S HOSPITAL LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2002/04/01-2004/03/31

DESCRIPTION (provided by applicant): Neonatal E. coli K1 meningitis is the most common serious infection of the central nervous system with unchanged rates of mortality and morbidity. Survivors of this disease suffer a number of complications including mental retardation and speech impairment. Limited knowledge about the pathogenesis and pathophysiology of this disease hampered the efforts to develop new therapeutic strategies for the prevention. For example, most cases of E. coli K1 meningitis occur via hematogenous spread, but it is unclear how the circulating E. coli evades the host-defense mechanisms. The investigator's studies have shown that outer membrane protein A (OmpA) of E. coli contributes to resistance to serum bactericidal activity. In addition, OmpA interacts with a brain specific 95 kDa receptor for E. coli invasion of the blood-brain barrier (BBB). The E. coli invasion of the BBB was significantly reduced in the presence of adult human serum (AHS) when compared to cord blood serum (CBS) using the investigator's in vitro model of the BBB, the cultured brain microvascular endothelial cells (BMEC). His data further showed that OmpA binds to C4-binding protein, a complement fluid phase regulator, in significant quantities from AHS when compared to CBS. A compelling observation is that the binding of C4-binding protein to OmpA blocked the E. coli invasion of BMEC, suggesting that it is competing with the OmpA-receptor. The investigator hypothesized that binding of C4BP to OmpA blocks the E. coli invasion of BMEC and that low levels of C4BP may contribute to the susceptibility of neonates to E. coli meningitis. He will pursue this hypothesis by study of the following specific aims. 1. To determine the binding site of C4BP on OmpA that blocks E. coli invasion of BMEC, and 2. To assess the effect of anti-OmpA antibody, OmpA-peptides, and C4BP-peptides on E. coli invasion of BMEC in the newborn rat model of hematogenous meningitis.

**Grant:** 1R03HD042133-01  
**Program Director:** SPONG, CATHERINE  
**Principal Investigator:** ROSS, ROBIN A PHD  
**Title:** Role of Bacterial Vaginosis in Preterm Delivery  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2002/04/01-2004/03/31

DESCRIPTION (provided by applicant): Bacterial vaginosis (BV) results from a disruption of the vaginal ecosystem characterized by a complex shift in the microflora. Concentrations of the normally dominant *Lactobacillus* decrease while other microflora increase (*Prevotella*, *Peptostreptococcus*, *Gardnerella*, *Mobiluncus*). Studies have linked BV with upper genital tract infections and adverse pregnancy outcomes, particularly preterm delivery (PTD). Bacteria can weaken fetal membranes through production of soluble factors that lead to PTD either by induction of a proinflammatory response or stimulation of prostaglandin E2 production. An inflammatory response leading to PTD can also be stimulated in host cells directly through attachment and internalization of bacteria. Our laboratory has developed in vitro models of the vaginal ecosystem that combine mixed cultures of normal (NMVF) and BV-associated (BVAf) bacteria with immortalized cervical and vaginal epithelial cells in coculture. Preliminary studies demonstrate these are viable models for studying bacterial-epithelial interactions of the vaginal ecosystem. Data from studies using NMVF and both cell lines indicate that all microflora components adhere to the epithelial cells, but only *Lactobacillus* (La), *Prevotella* (Pb), and *Enterococcus* are internalized. In addition, Pb stimulated interleukin-8 production while La induced significant apoptosis in cocultures. This application is divided into 2 aims designed to analyze the role of BV-associated microflora (BVAf) in the pathogenesis of PTD using these unique models. The inflammatory response to coculture will be characterized, comparing findings using NMVF and BVAf. Adherence, internalization, and apoptosis rates will be determined for BVAf and NMVF and compared. Bacterial factors produced during coculture will be determined and correlated with stimulation of an inflammatory response. Due to the novelty of the models, we have the unique opportunity to determine the role of BVAf in PTD.



**Grant:** 1R21HD042980-01  
**Program Director:** NEWCOMER, SUSAN  
**Principal Investigator:** COHEN, DEBORAH MD  
**Title:** ALCOHOL OUTLETS, BROKEN WINDOWS, GONORRHEA AND HIV RISK  
**Institution:** RAND CORPORATION SANTA MONICA, CA  
**Project Period:** 2002/07/01-2004/06/30

DESCRIPTION (provided by applicant): The 1992 Civil Unrest in Los Angeles resulted in the burning of more than 600 buildings and the closure of 221 alcohol outlets. These events serve as a natural experiment to test the influence of community institutions, [both alcohol outlets and community-based organizations-(CBOs)] on HIV risk in a longitudinal, ecological study. We propose to use longitudinal data of gonorrhea and HIV rates and neighborhood conditions to determine 1) if gonorrhea rates dropped in local neighborhoods where alcohol outlets were closed, 2) if there is an association between changes in neighborhood deterioration (or reconstruction) and changes in rates of gonorrhea, and 3) if there is an association between changes in alcohol outlets, neighborhood deterioration and changes in AIDS case rates at the census tract level. In addition, we propose to conduct a qualitative study of the efforts both prior to and since 1992 of CBOs to prevent the relicensure of alcohol outlets to assist in the interpretation of our quantitative study. Data on gonorrhea and HIV will be obtained from the LA County Department of Health Services. Addresses of destroyed buildings will be obtained from the Arson Section of the LA Fire Department. We have already obtained addresses of alcohol licenses that were surrendered in 1992, as well as existing alcohol licenses on an annual basis. All data will be geocoded to the level of the census tract and will be merged with data from the 1990 and 2000 US Census. Spatio-temporal modeling will be employed to determine whether changes in the community institutions (alcohol outlets) and neighborhood rehabilitation (or deterioration) are associated with GC and HIV rates, after controlling for a number of variables, including age, gender, race, socioeconomic status and preexisting rates of GC and HIV. The findings will inform future HIV prevention interventions and provide guidance to CBOs as to whether control of alcohol outlets and neighborhood development may enhance the prevention of HIV transmission.

**Grant:** 1R15HG002389-01A1  
**Program Director:** GOOD, PETER J.  
**Principal Investigator:** BROWN, DANIEL R PHD  
**Title:** Annotation of the *Mycoplasma alligatoris* genome  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 2002/09/30-2005/09/29

DESCRIPTION (provided by applicant): Mycoplasmas are important pathogens with usually strict natural host tropism and poorly understood virulence mechanisms. The broad objective of this work is increased understanding of mycoplasmal host-range and virulence determinants through comparison of conserved and variable features of mycoplasmal genomes. Comparative genomics is a new alternative to the usual tools of molecular biology to elucidate the genetic bases of mycoplasmosis. *Mycoplasma alligatoris* merits priority in that effort because in some hosts it causes hyperacute lethal disease, but it is merely commensal in others. The specific aims are to assemble a draft map of the *M. alligatoris* genome by alignment with that of closely-related *Mycoplasma pulmonis*, and to identify candidate genes involved in host tropism and virulence. The working draft will be assembled by using automated high-throughput sequence assembly methods. Sequence alignments will be validated by comparative synteny and PCR-based gap closure. Coding sequences will be identified by interpolated Markov models trained with mycoplasmal sequences and cataloged according to their public database matches. Candidate host-range genes may be recognized by overall similarity to known adhesins or by signature ligand-binding motifs. Virulence factors may include homologs to cell-surface or secreted hydrolytic enzymes of other mollicutes, or to pathogenic determinants present in other bacteria. The sequences of homologs whose known biological roles or predicted properties are consistent with host cell-surface ligand-binding or the pathogenic effects of *M. alligatoris* will be characterized in detail. The data will be integrated in a representation of the complement of adhesion, transport, and metabolic pathways of *M. alligatoris* validated by the biologically well-documented metabolic map of the mollicutes. The ease of PCR-based cloning and the accessibility of bioinformatics software make this an ideal project to engage undergraduate students in innovative biomedical research.

**Grant:** 1P01HL066196-01A1  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** CHRISTMAN, BRIAN W MD  
**Title:** Liver Lung Interactions in Lung Inflammation  
**Institution:** VANDERBILT UNIVERSITY NASHVILLE, TN  
**Project Period:** 2002/01/01-2006/11/30

It is abundantly clear from both experimental observations and clinical experience, that interactions between the liver and the lungs can play a critical role in the initiation and outcome of lung inflammation. We propose a Program Project consisting of four inter-related projects and three core areas to investigate mechanisms of interactions between the liver and the lungs in acute lung injury syndromes. The underlying theme, addressed in different by each of the projects, is that hepatic injury, whether resulting from endotoxemia, direct trauma or drug injury, can signal an inflammatory response in the lungs that may eventuate in acute lung injury and that eicosanoids play a critical role in modulating that process. Pro-inflammatory cytokines, including tumor necrosis factor alpha, produced in the liver, comprise the liver inflammatory signal. Activation of two transcription factors, nuclear factor kappa B (NFkB) and CCAAT enhancer binding protein beta (C/EBPbeta a.k.a. NF-IL6) is critical to the response in the lungs and in the liver and the course of the response of the lungs to liver injury is determined by the pattern of activation of these two factors. Oxidant stress is common to many causes of hepatic injury and abnormalities in function of the hepatic urea cycle exaggerate oxidant stress resulting in enhanced activation of transcription factors and thus cytokine release, amplifying the inflammatory signal to the lungs. The program forms a structural basis for enhancing previous and existing collaborations among investigators from Pulmonary Medicine, Surgery, Pediatrics, Pharmacology, Biochemistry, Microbiology and Immunology and Molecular Physiology and Biophysics. The program as a whole will integrate the pathophysiology of lung injury with biochemical cellular and molecular mechanisms of liver-lung relationships that contribute to or moderate the inflammatory response. Experimental approaches include whole animal physiologic preparations as well as numerous genetically altered mouse models and bone marrow and hepatocyte transplantation technology as experimental tools. The proposal promises to obtain new information related to mechanisms of lung injury which will be clinically relevant by providing rationales for new preventive or therapeutic strategies as well as the ability to identify at risk individuals. The proposal results from the conviction by the involved investigators that the common themes, complementary expertise and unique technologies assembled into a coordinated program will be more creative, more productive and more likely to advance understanding of the pathogenesis and potential therapy of inflammatory lung disease.

**Grant:** 2R01HL026148-23  
**Program Director:** CROXTON, THOMAS  
**Principal Investigator:** TRAVIS, JAMES  
**Title:** PROTEOLYTIC ENZYMES AND INHIBITORS IN LUNG DISEASE  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 1982/01/01-2006/03/31

DESCRIPTION (provided by applicant): Host proteolytic enzymes are believed to play a central role in the pathogenesis of pulmonary emphysema, through degradation of alveolar connective tissue proteins. However, little is known about whether this lung disease can be either caused or exacerbated by proteinases secreted by bacterial or fungal respiratory pathogens. Significantly, none of these enzymes are known to be regulated by host proteinase inhibitors. While it is believed that their primary function is to degrade host proteins to provide nutrients for the growth and proliferation of the invading organism, we propose that they also provide a means for evasion of host defense. For these reasons, the specific aims of this project are as follows: 1) to isolate and characterize selected proteinases secreted by lung pathogens, including *Aspergillus fumigatus*, *Stachybotrys chartarum*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, 2) to investigate the effect of pathogen-derived proteinases on the degradation/inactivation of host bactericidal peptides and proteins utilized to maintain homeostasis within the lung, and 3) to study the effect of exposure to these proteinases on a) the responsiveness of human monocytes and neutrophils to major pro-and anti-inflammatory stimulation and b) the ability of proteinase-exposed monocytes to clear apoptotic neutrophils. Our long-term goals are to determine whether the proteinases to be investigated play major roles in host defense evasion and tissue destruction within the lung. If this is the case, then they might be considered as targets for the development of inhibitors in order to control or eradicate lung microbial infections.

**Grant:** 2R01HL036611-14  
**Program Director:** MASSICOT-FISHER, JUDITH  
**Principal Investigator:** SCHLIEVERT, PATRICK M PHD  
**Title:** Cardiotoxicity of Streptococcal Pyrogenic Exotoxins  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 1986/07/01-2007/03/31

DESCRIPTION (provided by applicant): The long term goals of this project are two fold: a) to evaluate the role of pyrogenic toxin superantigens, notably streptococcal pyrogenic exotoxins (SPEs, scarlet fever toxins, in causing both acute toxic shock syndrome and vascular illnesses and chronic autoimmune and allergic diseases, and b) to analyze the structure:function relationships among the SPEs and between the SPEs and the staphylococcal enterotoxins and toxic shock syndrome toxin-1, with the intent of clarifying the molecular mechanisms of action of the toxins, developing toxoid vaccines, and developing useful adjuvants of the toxins. Specific aims of the present application include: a) Biochemical and immunobiological characterization of SPEs J and L, and determining the three dimensional structure of both toxins (complex structures of the SPEs with the variable part of the beta chain of the T cell receptor and major histocompatibility complex II molecules will be determined if such structures are likely to generate new data). Our role in this aim will be to characterize the new SPEs, provide toxins for structural studies, consult on the best conditions for use in crystallization, and preparation and testing mutant toxins for confirmation that important contact residues on the SPEs are required for activity; b) Characterization of SPE C's, and possibly SPE J's ability to cross mucosal surfaces. Studies will include establishment of vaginal epithelial monolayers and stratified epithelium in Transwells and evaluation of the mechanism by which the toxin(s) traverse the layers. We will also evaluate the ability of biologically inactive toxins to permeabilize the epithelium, both in vitro and in rabbits, to other agents, and thus, determine whether the toxoids may be useful as delivery agents (and possibly adjuvants) for transmucosal immunization; and c) Characterization of the mechanism of streptococcal toxic shock syndrome with necrotizing fasciitis in rabbits. We hypothesize that SPEs cause both hypotension and delayed phagocytosis through exaggerated cytokine release, which in turn allows continued growth of the invasive organism with production of necrotizing fasciitis through hemolysins and protease.

**Grant:** 2R01HL047569-11A1  
**Program Director:** GOLDMAN, STEPHEN  
**Principal Investigator:** BEASLEY, DEBBIE S PHD  
**Title:** Autocrine Role of Cytokines in Vascular Smooth Muscle  
**Institution:** NEW ENGLAND MEDICAL CENTER BOSTON, MA  
HOSPITALS  
**Project Period:** 1992/02/01-2005/11/30

Recent evidence suggests that chronic infection with the respiratory pathogen, Chlamydia pneumoniae, may contribute to the development of atherosclerosis. The long range objective of this proposal is to determine the mechanisms by which infection may contribute to enhanced vascular smooth muscle cell (VSMC) proliferation, a component of early atherosclerotic lesions. The working hypothesis for these studies is that VSMC recognize and respond to cell wall components of C. pneumoniae with a strong proliferative response and upregulation of interleukin-1alpha (IL1alpha) synthesis, which in turn amplifies and sustains the initial mitogenic signal elicited by chlamydial products. Studies during the previous grant period showed that the precursor form of IL1alpha is a potent membrane-associated growth factor for human VSMC (HVSMC), and that IL1-induced proliferation involves activation of TRAF6, NIK, and IKKs, leading to the persistent activation of NF-kappaB. Preliminary data indicate that a heat-labile component of C. pneumoniae is a potent mitogenic stimulus for HVSMC. The proposed studies will determine the mechanisms of C. pneumoniae-induced HVSMC proliferation, focusing on the role of Toll-like receptor 4 (TLR4), which is expressed by HVSMC. TLRs mediate recognition of bacterial products, including lipopolysaccharide and heat shock protein 60, have intracellular domains which are homologous to the type I IL1 receptor, and likewise activate TRAF6, NIK and IKKs. Two specific hypotheses will be tested in the proposed studies. First, C. pneumoniae induces HVSMC proliferation via activation of TLR4, with subsequent recruitment of TRAF6 and ultimate activation of NF-kappaB and p44/p42 mitogen-activated protein kinases. Second, autocrine production of IL1alpha, and its myristylation-dependent localization to the cell surface, sustains and enhances the primary effects of TLR4 activation. The specific aims are: to determine whether C. pneumoniae or its molecular components induce proliferation of HVSMC via TLR4-mediated activation of TRAF6, NIK, IKK, and ultimately NF-kappaB, and by TRAF6-mediated activation of p42/p44 mitogen-activated protein kinases; to determine whether autocrine production of IL1alpha precursor contributes to the mitogenic effect of C. pneumoniae in HVSMC; and to determine whether myristylation of lysine83 plays a crucial role in the mitogenic effects of IL1alpha precursor by targeting it to the plasma membrane. The studies will employ transient transfection with dominant negative mutants, TLR4 and IL1 receptor antagonists, and antisense oligonucleotides. The results of these studies will elucidate the potential roles of bacterial and chlamydial products in the pathogenesis of vascular disease.

**Grant:** 2R01HL048540-08A2  
**Program Director:** BAROUCH, WINIFRED  
**Principal Investigator:** SHECHTER, ISHAIAHU PHD  
**Title:** Cytokine-Mediated Regulation of Cholesterologenesis  
**Institution:** HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD  
MIL/MED  
**Project Period:** 1993/06/01-2006/06/30

DESCRIPTION (provided by applicant): Squalene synthase (SQS) catalyzes the first reaction of the isoprenoid metabolic pathway committed to cholesterol biosynthesis and its activity regulates the flux of intermediates to sterols. SQS is regulated by sterols, lipopolysaccharide (LPS), and the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-alpha) and interleukin-1-beta (IL-1-beta). Sterol-mediated transcriptional regulation of SQS is well understood, but little is known about the mechanism of its regulation during the inflammatory response. We propose to test the hypothesis that LPS and cytokines regulate SQS mRNA transcription and stability and enzyme protein posttranslational modification, stability and subcellular localization. In Specific Aim 1 we will determine the effects of LPS and cytokines on hepatic SQS transcription and mRNA stability in Syrian hamsters fed with different cholesterologenic diets. We will also determine changes in SQS mRNA level and size during response to cytokines and the contribution of the specific cytokines to these processes. In Specific Aim 2 we propose to elucidate the molecular mechanisms responsible for SQS mRNA regulation by LPS and cytokines. This will be achieved, in cultured cells, by examining promoter sequence elements responsible for the transcriptional repression by cytokines, by identification of transcription factors involved in this suppression, and by the elucidation of cellular signaling pathways involved in the transcriptional regulation. In addition, sequences required for SQS mRNA destabilization by cytokines will be localized. In Specific Aim 3 we propose to elucidate the LPS-and cytokine-induced, post-translational mechanisms underlying the decrease in SQS enzymic activity. We will examine SQS protein destabilization, mechanisms for its degradation, post-translational modification by phosphorylation, and change in its subcellular localization in response to LPS and cytokines. Achievement of the three Specific Aims will contribute directly to our long-term goal to elucidate the importance of SQS regulation on isoprenoid metabolic flux. It will increase our understanding of sterol metabolism in acute phase response (APR) to infection and inflammation and the role of SQS regulation in this process. Finally, it will enhance our overall understanding of hepatic cholesterol production in normal and pathological situations.

**Grant:** 2R01HL048872-10A1  
**Program Director:** HASAN, AHMED A.K.  
**Principal Investigator:** MACKMAN, NIGEL PHD GENETICS, OTHER  
**Title:** Regulation of inducible gene expression in monocytes  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 1992/07/01-2006/03/31

Gram-negative sepsis is a major cause of death in intensive care units in the United States. Sepsis is induced by the presence of pathogenic bacteria in the blood. Monocytes of the host innate immune system orchestrate a rapid response to bacterial lipopolysaccharide (LPS [endotoxin]) by expressing various cytokines and by expressing the procoagulant protein tissue factor (TF), which initiates disseminated intravascular coagulation. Recent studies indicate that administration of anticoagulants reduces mortality in patients with severe sepsis. The long-term objectives of this proposal are to elucidate the mechanism by which coagulation proteases contribute to inflammation during sepsis. Our central hypothesis is that FXa activation of protease activated receptor 2 (PAR-2) enhances IL-6 expression and increases lethality during sepsis. We will also determine the role of thrombin (FIIa) -PAR- 1 signaling in sepsis. We will employ selective inhibitors of FXa and FIIa and analyze PAR-1- and PAR-2-dependent mice in a lethal mouse model of sepsis. In addition, we will use bone marrow transplantation to determine the role of TF and PAR-1 expression on monocytes versus endothelial cells in sepsis. Finally, we will generate mice that constitutively or inducibly express PAR-2 in endothelial cells to directly test our central hypothesis that PAR-2 is a key component of a pathogenic pathway involved in lethal sepsis. These studies should define the mechanism by which coagulation proteases enhance inflammation during sepsis. The clinical relevance of these studies is that they may provide new insight that can be used to develop improved therapeutic strategies for the treatment of patients with severe sepsis.



**Grant:** 2R01HL049040-11A1

**Program Director:** BANKS-SCHLEGEL, SUSAN P.

**Principal Investigator:** WILSON, JAMES M MD  
BIOCHEMISTRY:BIOCHEM  
RY-UNSPEC

**Title:** Xenograft Model of Cystic Fibrosis Therapy

**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA

**Project Period:** 1994/09/30-2006/03/31

DESCRIPTION (provided by applicant): Studies of the pathogenesis of cystic fibrosis (CF) have highlighted the importance of host defenses at the airway surface in combating microbial infections. The airway epithelial cell plays a key role in pulmonary host defense through a number of different pathways. During the last cycle of this grant, we identified and characterized from humans and mice novel molecules, called beta-defensins, which are secreted by epithelial cells on to the surface of the airway where they contribute to bacterial killing. Mice individually deficient in two mouse beta-defensin genes were created and subjected to preliminary analysis. Recent work by others has determined a family of transmembrane proteins called Toll Like Receptors (TLRs) important signaling innate immunity and initiating acquired immune responses. The goal of this proposal is to evaluate the role of the pulmonary epithelia in initiating the innate immune response in murine models to two pathogens important to the pathogenesis of CF, H. influenzae and P. aeruginosa. In preclinical studies, we document expression of human TLR in airway epithelia cells and its role in signaling innate immunity. Studies in mice challenged with H. influenzae documented the importance of murine beta-defensin 1 in clearing the infection and TLR4 in sensing the infection and signaling an innate immune response. Studies with P. aeruginosa suggest a critical role of TLR4 in the effector response of innate immunity. Specific Aim 1 will evaluate the relative role of the epithelia versus macrophages in sensing and signaling innate immunity and will attempt to confirm/identify specific TLRs involved in detecting the pathogens under study. Specific Aim 2 will characterize the role of mBD- 1 and mBD-3 in responding to intrapulmonary challenge of prototypic pathogens. Specific Aim 3 will delineate the proposed defect(s) in effector cell function to P. aeruginosa observed in TLR deficient mice.

**Grant:** 2R01HL054972-06A1  
**Program Director:** COLOBINI-HATCH, SANDRA  
**Principal Investigator:** SKERRETT, SHAWN J MD  
**Title:** Host Defense Against Intracellular Infection of the Lung  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1996/04/10-2006/11/30

DESCRIPTION (provided by applicant): Legionella pneumophila is the cause of Legionnaires' Disease and a prototypical intracellular parasite of alveolar macrophages. The pathways involved in the initial recognition of inhaled bacteria are likely to be critically important in the activation of innate defenses and the initiation of specific adaptive immunity. The signaling receptors that mediate early responses to L. pneumophila in the lower respiratory tract and the mechanisms by which this organism subverts the defensive response to infection are poorly understood. Toll-like receptors have emerged as an important family of pattern recognition molecules that can initiate cellular activation responses to a wide variety of microbial stimuli. The roles of Toll-like receptors in mediating alveolar macrophage responses to infection and in activating pulmonary anti-bacterial defenses are unknown. Virulent L. pneumophila is able to blunt the cytokine response of alveolar macrophages by unelucidated mechanisms that are dependent on the expression of specific bacterial genes. The overall goals of this proposal are to determine the recognition pathways that stimulate innate defenses to intracellular bacteria, and to explore mechanisms by which virulent organisms subvert host resistance. The specific aims are as follows: 1. Determine the roles of Toll-like receptors (TLRs) in mediating cellular responses to L. pneumophila (Lp). This aim will test the hypothesis that TLRs, singly or in combination, mediate the initial activation of alveolar macrophages in response to Lp, serving to defend the cell against intracellular infection. 2. Determine the roles of TLRs in pulmonary host defense against Lp in vivo. This aim will test the hypothesis that signaling via TLRs serves to stimulate innate defenses against Lp that control early bacterial clearance and initiation of the adaptive response. 3. Define the mechanisms by which virulent L. pneumophila subverts the activation response of alveolar macrophages. This aim will test the hypothesis that bacterial dot/icm genes direct macrophage uptake of L. pneumophila by a pathway that results in diminished cellular activation in comparison bacteria deficient in these loci.

**Grant:** 2R01HL056194-05  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** PRINCE, ALICE S  
**Title:** Bacterial Induction of Cytokine Expression in the Lung  
**Institution:** COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY  
**Project Period:** 1997/12/20-2005/11/30

DESCRIPTION (provided by applicant): Airway epithelial cells provide a major immunological function, signaling the presence of potential infectious agents in the airway lumen. The normal function of this surveillance system keeps the airways pristine, whereas abnormalities in epithelial signaling and inflammatory responses contribute significantly to the development of chronic inflammation, characteristic of cystic fibrosis. Epithelial cells are activated by specific bacterial gene products and NF-kB dependent transcription of chemokines and cytokines follows promptly. In the studies proposed, we will examine the molecular mechanisms involved in the immediate response of the epithelium to bacterial gene products and the signaling pathways that accomplish this function. We postulate that common receptor complexes are mobilized to the epithelial cell surface in response to shed bacterial components, activating responses even in the absence of bacteria adherent to the epithelial surface. TLR2, a major receptor for several conserved bacterial components, is co-localized with asialylated glycolipid receptors and similarly mobilized to the cell surface by both gram negative and gram positive pathogens. The first aim of this project will be to establish the biology of TLR2 in the airway epithelium. To assess the role of TLR2 in airway cells, we will construct a bi-transgenic mouse which has TLR2 expression limited to the lung, to differentiate the contribution of the epithelial cell in signaling the early stages of infection, from the function of "professional" immune cells, alveolar macrophages and T cells. A comparison of the responses of TLR2 null mice, wild type mice, and mice with TLR2 expression limited to the lung, to both virulent, invasive *S. aureus*, as opposed to non-invasive mutants which cause pneumonia in CF, but minimal mortality will be done. In the second aim, we will examine the interactions of *S. aureus* and the airway epithelial cell, focusing specifically on the ability of staphylococcal gene products particularly peptidoglycan and protein A in the activation of the epithelial cells, and their specific activation of TLR2 associated signaling pathways. The role of TLR2 in normal airway epithelial cells and cells with CFTR dysfunction will be compared to determine if excessive activation or impaired regulation of this signaling cascade is associated with the excessive inflammatory response characteristic of cystic fibrosis.

**Grant:** 2R01HL057243-06  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** STANDIFORD, THEODORE J MD INTERNAL  
MED/PULMONARY MED  
**Title:** ELR-CXC Chemokines In Lung Antibacterial Host Defense  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1996/12/01-2005/11/30

DESCRIPTION (provided by applicant): Bacterial pneumonia is the second most common cause of hospital-acquired infection, and is leading cause of death among all nosocomial infections. Innate, or natural immunity, is the principal pathway for effective elimination of bacterial organisms from the lung. While ELR-CXC chemokines have been shown to be expressed during the generation of Th1 cell-mediated immune responses against intracellular microbial pathogens, the contribution of this family of chemokines to innate immunity against common gram-positive and gram-negative bacterial pathogens is unknown. We have focused this competitive renewal on ELR-CXC chemokines, as our preliminary observations indicate that the in-vivo depletion of selected ELR-CXC chemokines substantially impairs bacterial clearance and survival of mice with pneumonia due to *Klebsiella pneumoniae*. It is the hypothesis of this proposal that ELR-CXC chemokines are integral components of the innate neutrophil-dependent immune response against gram-negative bacterial infection of the lung. A murine model of *Klebsiella pneumoniae* will be employed to achieve the following specific aims: 1) to determine the time course of expression and cellular sources of ELR-CXC chemokines and their common receptor (CXCR3) during the evolution of gram-negative bacterial pneumonia; 2) to determine the contribution of ELR-CXC chemokines and their receptor to leukocyte recruitment, proinflammatory cytokine expression, bacterial clearance, survival in *Klebsiella pneumoniae* using specific neutralizing antibodies or knockout mice; 3) to determine the effect of IP-10 or MIG administration/transgenic expression on proinflammatory cytokine expression, bacterial clearance, and survival in murine *Klebsiella pneumoniae* in-vivo and on alveolar macrophage effector cell function in-vitro; and 4) to identify endogenous signals that regulate the expression of ELR-CXC chemokines during the evolution of *Klebsiella pneumoniae* in-vivo and in isolated lung cells in-vitro. Elucidation of specific cellular and molecular mechanisms of lung antibacterial host defense, in conjunction with the use of novel gene therapy approaches will provide important insights into the treatment of patients with serious multi-drug resistant bacterial infections of the lung.

**Grant:** 2R01HL058334-05  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** WOZNIAK, DANIEL J PHD MICROBIOLOGY, OTOLARYNGOLOGY  
**Title:** Control of *Pseudomonas aeruginosa* algD transcription  
**Institution:** WAKE FOREST UNIVERSITY HEALTH SCIENCES WINSTON-SALEM, NC  
**Project Period:** 1996/09/30-2006/06/30

DESCRIPTION (provided by applicant): Cystic fibrosis (CF) patients are predisposed to recurrent respiratory tract infections by the bacterium *Pseudomonas aeruginosa*. Complications arising from these infections are a major source of morbidity and the leading cause of death in those afflicted. *P. aeruginosa* strains that initially colonize the lungs are nonmucoid, but over time mucoid variants emerge and this is correlated with a worsening clinical condition for the CF patient. The mucoid phenotype is due to high-level synthesis of a capsular polysaccharide called alginate and overproduction of this virulence factor confers a selective advantage for *P. aeruginosa* in the CF lung. Thus, the long-term objective of this proposal is to understand the molecular mechanisms responsible for the production of alginate by strains of *P. aeruginosa* which colonize CF patients. Most of the genes for alginate production are in a large operon that is transcribed by a tightly controlled promoter (*palgD*). *palgD* is activated in mucoid *P. aeruginosa* isolates but no transcription is detectable from this promoter in nonmucoid strains. This proposal will focus on AlgZ and AlgB, two proteins that are essential for *algD* activation. In addition, experiments aimed at defining the events leading to mucoid conversion and subsequent *algD* activation are proposed. Biochemical and genetic approaches will be utilized to address three central questions which constitute the basis of this proposal: (1) What is the mechanism of AlgZ-mediated transcriptional activation of the *algD* promoter? (2) How does the response regulator AlgB controls *algD* expression and alginate synthesis? (3) What are the molecular events controlling mucoid conversion in *P. aeruginosa*? Since the overproduction of alginate correlates with a poor clinical outcome for CF patients colonized with mucoid *P. aeruginosa*, and since *algD* activation is a prerequisite for alginate synthesis, a basic understanding of *algD* transcription is essential for understanding the pathogenesis of *P. aeruginosa*. This will lead to novel therapies and improve the quality of life for CF patients colonized with mucoid *P. aeruginosa*.

**Grant:** 2R01HL058398-05  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** PIER, GERALD B PHD MICROBIOLOGY, OTTAWA  
**Title:** CFTR and Infection of Cystic Fibrosis patients  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 1999/07/01-2005/11/30

DESCRIPTION (provided by applicant): The long-term goal of this project is to understand the molecular and cellular basis of the hypersusceptibility of cystic fibrosis (CF) patients to chronic infection with *Pseudomonas aeruginosa*. This infection is responsible for greater than 80 percent of the morbidity and mortality that occurs in CF patients. A key component of this hypersusceptibility is the role and function of the cystic fibrosis transmembrane conductance regulator (CFTR) in host resistance to *P. aeruginosa*. CFTR in epithelial cell membranes binds directly to *P. aeruginosa* and mediates bacterial internalization, an interaction critical for microbial clearance in individuals with wild-type CFTR. Although the major components of the ligand-receptor interaction have been identified the work proposed in this application will explore more of the complexities of this interaction. One focus will be on bacterial factors that provoke the cellular response leading to ingestion of whole *P. aeruginosa* cells and LPS. A second focus will be on the CFTR-dependent eukaryotic cellular responses to *P. aeruginosa* infection. The first set of aims will encompass: a) identification of *P. aeruginosa* proteins that provoke translocation of CFTR from cytoplasmic stores to plasma membranes, by identifying and measuring the ability of purified bacterial products to provoke membrane localization of CFTR and determining their role in the pathogenic process using cell culture and animal models of infection; b), a detailed structural analysis of the chemical components of the *P. aeruginosa* LPS involved in binding to CFTR using NMR and mass spectrometry techniques. The second aim will focus on the cellular activation and signaling molecules elicited in response to the *P. aeruginosa*-CFTR interaction and their role in host resistance to infection. Specific areas of investigation encompass: a) studies using FACS and confocal microscopy on how CFTR extracts and internalizes the LPS from the bacterial outer membrane leading to NF-kappa B and other eukaryotic cellular responses critical for coordination of innate immunity; b) the molecular and genetic factors involved in progression of cells with wild-type CFTR to apoptosis which does not occur comparably in CF cells; and c) the role of the Fas-Fas Ligand system in CFTR-controlled apoptosis and resistance to *P. aeruginosa* infection. From these studies we anticipate ascertaining how wild-type CFTR coordinates immunity to *P. aeruginosa* lung infection and how this process is defective in CF patients. Such insights may lead to interventions to prevent the common occurrence of chronic *P. aeruginosa* infection in CF patients.

**Grant:** 2R01HL058496-05  
**Program Director:** HASAN, AHMED A.K.  
**Principal Investigator:** REED, GUY L MD  
**Title:** Plasminogen Activation & SK: Structure-Function  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 1998/07/01-2006/06/30

DESCRIPTION (provided by applicant): The plasminogen (Pg) system dissolves the thrombi (blood clots) that cause heart attacks and strokes. The Pg system is tightly regulated by protein-protein interactions with inhibitors, activators, substrates, etc. The cleavage of Pg to plasmin by streptokinase (SK), and other Pg activators, initiates fibrinolysis (clot dissolution) which saves the lives of heart attack patients. Recent studies have suggested that mechanistic insights into the regulation of the Pg system could further reduce the mortality from heart attacks, and improve the treatment of strokes, pulmonary embolism, etc. Because of its physiologic and therapeutic importance, our long term goal is to help elucidate the protein-protein interactions that regulate and modify the activity of the Pg system. The interactions between Pg and the indirect Pg activator SK are among the most biologically and medically important of these contacts. Studies performed in the first phase of this grant have helped to delineate the elegant interactions through which SK converts Pg (without cleavage) into the most catalytically efficient Pg activator, Insights have been made into defining the mechanisms through which: 1) SK forms a tight stable 'activator complex' with Pg (or plasmin), 2) SK non-proteolytically generates the latent active site in Pg creating a 'virgin enzyme' (Pg\*), and 3) SK modifies the substrate specificity of Pg\* or plasmin so that the complex can cleave Pg molecules. This continuation proposal is directed towards further dissecting the process of indirect Pg activation, in order to determine the novel mechanisms by which SK becomes a fibrin-dependent (or t-PA-like enzyme), to define whether fibrin-dependent SKs have the potential to be superior fibrinolytic agents, to understand the role of the Pg kringle domains in indirect Pg activation and, to define the intermolecular interactions that occur in the SK-Pg complex which are required for a SK-type of mechanism. In a broad scientific sense, insights into this unique process of indirect Pg activation should enlarge our understanding of how the catalytic activity and specificity of Pg system is regulated, and could suggest rational ways to alter the indirect Pg activators so as to improve their therapeutic value for patients with thrombosis.

**Grant:** 2R01HL059836-06  
**Program Director:** PEAUVY, HANNAH H  
**Principal Investigator:** KRAMNIK, IGOR MD  
**Title:** Genetics of Host Resistance & Susceptibility to TB  
**Institution:** HARVARD UNIVERSITY (SCH OF PUBLIC HLTH) BOSTON, MA  
**Project Period:** 1997/09/30-2007/07/31

DESCRIPTION (provided by applicant): A significant variation in susceptibility to tuberculosis among immunocompetent individuals is partially explained by genetic heterogeneity within the host population. However, precise mechanisms of the genetic control of anti-tuberculosis immunity are unknown. We employ a mouse experimental model of tuberculosis for the genetic analysis of the naturally occurring variation in tuberculosis resistance among immunocompetent inbred mouse strains to identify and isolate genes important for determining susceptibility to this infection. We have previously mapped the *sst1* locus, which controls progression of pulmonary tuberculosis early after infection. Using the *sst1*-resistant congenic mice we mapped six new quantitative trait loci (QTL) that control variation in tuberculosis resistance among the *sst1*-resistant hosts. Functional expression of some of those loci is *sst1*-dependent. We will study the new loci and characterize their possible interactions using genotype-assisted breeding and advanced backcross-intercross progeny testing in order to narrow candidate intervals and establish conditions for their further genetic dissection. We will generate a set of congenic strains by transferring new candidate QTLs on a C3H background and dissect those loci into smaller chromosomal segments to facilitate positional cloning. Using new congenic strains we will study cells that form lung granulomas in resistant and susceptible animals. Identify correlates of tuberculosis resistance and susceptibility at molecular level and establish proxy phenotypes for each locus that can be used as surrogate biomarkers for predicting genetic susceptibility in segregating populations. Identification of molecules encoded in QTLs will help explain critical functional differences conferred by the genetic polymorphisms on mechanisms of host resistance to tuberculosis at both systemic and lung-specific levels.



**Grant:** 2R01HL059838-06A1  
**Program Director:** PEAUVY, HANNAH H  
**Principal Investigator:** GOLDFELD, ANNE E MD  
**Title:** GENETIC FACTORS AND SUSCEPTIBILITY TO TUBERCULOSIS  
**Institution:** CBR INSTITUTE FOR BIOMEDICAL RESEARCH BOSTON, MA  
**Project Period:** 1997/09/30-2007/07/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) is the leading opportunistic cause of death among AIDS patients and is responsible for the loss of almost 3 million lives each year. Studies from our laboratories have associated a specific family of HLA alleles (HLA-DQ beta57-Asp) with elevated risk for progression to active pulmonary disease among a group of Cambodian rural poor suffering from one of the highest global incidences of TB. We have also uncovered a high incidence of antigen-specific anergy to PPD (purified protein derivative) among patients with active pulmonary disease that persists following successful cure. This persistent anergy may be associated with the same HLA type. The proposed studies will expand our initial assessment of HLA alleles with disease and anergy, attempt to establish the clinical relevance of anergy and HLA type in TB, explore the molecular mechanisms involved in anergy at the transcriptional level, and explore the role of antigen-specific anergy and different immunomodulatory TB strains in HIV-1 regulation. In Specific Aim 1 we propose to (1) solidify the correlation of HLA-DQ ?57-Asp with anergy and susceptibility to TB by HLA-typing additional anergic patients in Cambodia, (2) compare the HLA type of HIV/AIDS patients suffering from TB with the HLA type of those that avoid TB infection to uncover more subtle effects in this highly vulnerable population, (3) determine the functional consequences of anergy by looking at the rate of bacterial clearance in anergic TB patients undergoing antituberculosis chemotherapy, (4) determine the functional consequences of anergy by HLA typing patients that experience a poor clinical outcome and relapse with active disease following apparently successful chemotherapy and (5) determine whether HLA-DQ ?57-Asp effects the presentation of Mtb-derived antigens. In Specific Aim 2 we propose to (1) study the molecular mechanism of anergy in the infected host by exploring the nature of chromatin structure and remodeling in T-cells from PPD-anergic patients, and (2) determine the role of specific transcription factors in anergy. In Specific Aim 3 we will examine the impact of PPD-driven T cell activation in the setting of HIV-1 infection and we will determine whether monocyte tropic as well as T cell tropic primary HIV-1 isolates are regulated differently in PPD-responsive versus PPD-anergic cells. Furthermore, we will clarify the role of different immunomodulatory MTb strains in the regulation of T and M tropic HIV-1 primary isolates+ Finally, MTb strains from anergic and PPD-responsive patients will be isolated and molecularly characterized and will be tested for distinct effects upon T and M tropic primary HIV-1 isolates. Taken together, these experiments should allow us to elucidate basic mechanisms in MTb and HIV-1 coinfection and suggest novel immunomodulatory therapeutic strategies to decrease both viral load and MTb burden. Furthermore, these experiments will yield important basic insights into TB susceptibility and the molecular mechanisms of antigen-specific anergy. They may also contribute to our ability to predict treatment failure, prioritize patients for chemoprophylaxis, and design effective peptide vaccines.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 2R01HL060626-04A2  
**Program Director:** HASAN, AHMED A.K.  
**Principal Investigator:** ZHANG, XUEJUN C PHD  
**Title:** Molecular Basis of Plasminogen-Streptokinase Interaction  
**Institution:** OKLAHOMA MEDICAL RESEARCH OKLAHOMA CITY, OK  
FOUNDATION  
**Project Period:** 1998/07/06-2006/06/30

DESCRIPTION (provided by applicant): Plasminogen activation is the central event in fibrinolysis, which is of importance to the strategy of short term treatments of acute thrombolytic disorders. Plasminogen activation also plays critical roles in cell migration related to tumor growth and metastasis, Alzheimer's disease and related cerebral hemorrhage and some pathogenic invasions. Streptokinase, a bacterial protein, is a plasminogen activator widely used in the clinical treatment of myocardial infarction and other clotting disorders. Unlike tissue plasminogen activator and urokinase, streptokinase is not a protease. Streptokinase and plasminogen form a non-covalent complex, which is proteolytically active and converts other plasminogen molecules to plasmin leading to fibrinolysis. Previously, we determined the crystal structures of the catalytic domains of plasminogen and plasmin and the structure of streptokinase. While these structures have provided significant insight into the plasminogen activation and interactions between streptokinase and plasminogen, they also raise new questions about the detailed mechanisms by which the plasminogen:streptokinase complex activates other plasminogen molecules and about the regulation of plasminogen activation by a variety of effectors. The current application proposes to address questions on the mechanism of nonproteolytic activation of plasminogen in the plasminogen:streptokinase complex, the interaction of the substrate plasminogen with the activator complex and interactions between plasminogen activation and some of its physiological/pathological effectors using mutagenesis and x-ray crystallographic approaches.

**Grant:** 2R01HL062624-04  
**Program Director:** ORTEGA, HECTOR G.  
**Principal Investigator:** PEDEN, DAVID B MD  
**Title:** Endotoxin and Bronchial Inflammation in Asthma  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NC  
HILL  
**Project Period:** 1999/01/01-2005/12/31

DESCRIPTION (provided by applicant): In earlier studies, we have observed that allergen inflammation, induced either by allergen challenge or by natural exposure to aeroallergens during pollination seasons, enhances neutrophil influx to airway mucosal tissues following endotoxin challenge. We have also found that pre-challenge expression of CD14 on airway macrophages correlates strongly with neutrophil influx following endotoxin challenge and with baseline levels of eosinophils in the airway, and the treatment with inhaled corticosteroids decreases baseline eosinophil and macrophage CD14 expression and blunts response to endotoxin. We have also observed that repeated challenge with endotoxin induces tolerance to endotoxin, a phenomenon that, in animals, is reversed by GM-CSF, a product of TH2 inflammation. These findings support the hypothesis that allergic inflammation enhances response to endotoxin. Our proposal to continue these studies focuses on in vivo studies in human volunteers to examine the effect of allergen-induced inflammation on airway macrophage expression of molecules important in mediating endotoxin responsiveness and the effect of allergen challenge on bronchial response to inhaled endotoxin. We will also focus on development of tolerance to endotoxin in the bronchial airway, and assessment of CD14, TLR4, IL-1 receptor associated kinase, I $\kappa$ B and other mediators or modulators of endotoxin signal transduction in airway macrophages from persons undergoing a tolerance-inducing endotoxin challenge. We will also employ nasal challenge and in vitro studies of monocytic cells to examine the effect of GM-CSF and other candidate cytokines produced by IgE-mediated inflammation on in vivo and in vitro response to LPS

**Grant:** 1R01HL065465-01A2  
**Program Director:** PETERSON, CHARLES M  
**Principal Investigator:** YEH, SYUN-RU PHD  
**Title:** Structure Function Relationship in Hemeproteins  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2001/12/01-2005/11/30

The objective of this proposal is to elucidate the protein-ligand interactions and structure/function relationships in three new bacterial hemoglobins (Hb) and two mammalian prostaglandin H synthases (PGHS-1 and PGHS-2). The two bacterial hemoglobins from *Mycobacterium tuberculosis* (HbN and HbO) belong to a newly discovered truncated hemoglobin family, which are characterized by a novel two-over-two alpha-helical sandwich motif, the absence of the A-helix and the presence of an extended loop substituting for most of the F-helix. The physiological functions of HbN and HbO are not established but because O<sub>2</sub> delivery in unicellular organisms is a diffusion-controlled process, functions other than oxygen transport have been put forth. The bacterial hemoglobin from *E. coli* (Hmp) is a flavohemoglobin consisting of a heme-containing globin-like domain and a FAD-containing reductase domain. It is believed that the function of Hmp is to detoxify NO and other reactive nitrogen species. The structural properties of the three bacterial hemoglobins will be fully characterized. Based on our preliminary resonance Raman studies, we postulate that the heme pockets of these bacterial hemoglobins are tailored to perform chemistry, such as oxygen activation, and that they may share structural and functional similarities to peroxidases. This hypothesis will be tested by studies of the reactions of these hemoglobins with NO, hydrogen peroxide and peroxynitrite. The possible role of these hemoglobins in protecting the microorganisms against attack by reactive nitrogen intermediates will be explored by monitoring NO and O<sub>2</sub> consumption in wild-type and hemoglobin knock-out cells. Related reactions will be studied in the peroxidase sites of two PGHS isoforms, which play an essential role in the synthesis of prostaglandins. Preliminary data suggests that the proximal bond that coordinates the heme to the polypeptide is quite weak in PGHS's, which is very unusual for peroxidases. Experiments are proposed to test the functional consequences of this finding. These hemeprotein systems provide an excellent model for investigating fundamental structural properties that underlie biological reactivity.

**Grant:** 1R01HL067211-01A1  
**Program Director:** GANGULY, PANKAJ  
**Principal Investigator:** PEERSCHKE, ELLINOR I PHD BOTANY NEC:BOTAN  
NEC-UNSPEC  
**Title:** gC1qR/p33 in S. Aureus Endovascular Pathogenesis  
**Institution:** WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY  
UNIV  
**Project Period:** 2001/12/01-2005/11/30

In addition to their well known role in hemostasis and thrombosis, platelets and endothelial cells participate in inflammation, and react with components of the immune system. Moreover, these cells express receptors for the early complement component C1q, which may function in innate immunity by mediating the phagocytosis of invading pathogens. Preliminary data suggest that gC1qR/p33, originally described as a binding site for the globular domain of C1q, recognizes both *Staphylococcus aureus* protein A (SPA) and fibrinogen. Thus, we hypothesize that gC1qR/p33 participates in both direct and indirect staphylococcal interactions with platelets and endothelial cells. Since *S. aureus* is a major etiologic agent of endovascular infections in humans, particularly infective endocarditis, understanding the molecular mechanisms involved in infection is of considerable importance. In vitro studies are proposed using human platelets, cultured human endothelial cells, and a CHO cell line expressing cell membrane gC1qR/p33 to characterize gC1qR/p33 mediated *S. aureus* adhesion to cell surfaces. SPA+ and SPA- isogenic strains of *S. aureus*, and isogenic *S. aureus* strains differing in their ability to adhere to and clump fibrinogen (ClfA+, ClfA-) will be tested to elucidate the involvement of SPA and fibrinogen in this process. Purified native and recombinant gC1qR/p33 and monoclonal and polyclonal anti gC1qR antibodies are available for competing with or blocking cell surface gC1qR/p33, respectively. Specific aims are proposed 1) to characterize gC1qR/p33 -SPA binding, 2) to identify gC1qR/p33 as a platelet and endothelial cell binding site for *S. aureus*, 3) to examine the effect of circulating IgG and C1q on SPA-gC1qR/p33 binding, 4) to assess the role of gC1qR/p33 in *S. aureus* induced platelet aggregation and endothelial cell infection, and 5) to evaluate the ability of gC1qR/p33 to modulate *S. aureus* interactions with fibrinogen/fibrin in the extracellular matrix or on cell surfaces. Since *S. aureus* interactions with platelets and endothelial cells are complex and multimodal, in vivo studies are proposed using a well characterized rabbit endocarditis model to assess the role of gC1qR/p33 in *S. aureus* adhesion to sterile vegetations, infective lesion progression, and metastasis. Results from these studies will define a novel mechanism for *S. aureus* interactions at sites of endovascular damage, and may identify novel targets for therapeutic intervention.

**Grant:** 1R01HL067221-01A1  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** HICKEY, ANTHONY J PHD  
**Title:** MECHANISMS OF RESPONSE TO PULMONARY VACCINATION  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2002/04/01-2006/03/31

**DESCRIPTION:** (Provided by Applicant) Given the notorious variability in the protective efficacy of the current tuberculosis (TB) vaccine, BCG, in diverse populations around the world, there is a clear need for an improved TB vaccine. Although a major scientific effort over the past several years has produced literally hundreds of potential TB vaccine candidates, very little research has gone into alternative delivery systems and new adjuvants which will be required for purified protein or peptide vaccines. More importantly, given the pulmonary route of exposure by which most TB patients acquire their primary infection, it is surprising that little data derived regarding aerosol vaccination can be found in the recent biomedical literature. There are many reasons, a priori, to expect that direct immunization of the lung would have intrinsic advantages over parenteral routes of vaccination. Delivery of immunogens and adjuvant to the alveolar spaces using a microparticle aerosols should elicit local immune responses, which are effective at controlling the early replication of virulent Mycobacterium tuberculosis (MTB). Expertise in microparticle formulation, pulmonary aerosol delivery, a guinea pig model of low-dose pulmonary MTB and guinea pig immunology will be employed to elucidate the mechanisms by which aerosol vaccination leads to protection against virulent challenge. It is proposed that: (1) Lung delivery of immunostimulatory adjuvants (Muramyl dipeptide and trehalose dimycolate) in microparticle formulations will upregulate proinflammatory and co-stimulatory functions in resident alveolar macrophages; (2) The combination of immunogenic mycobacterial proteins with the adjuvant in microparticles will induce a strong expression of local antigen-specific T and B lymphocyte responses in the lung; (3) Aerosol vaccination with the optimal combination of protein antigen (Ag 85 complex)/adjuvant/micro-particles will protect guinea pigs against low-dose pulmonary challenge with virulent MTB. The significance of this work lies in the novelty of pulmonary vaccination for the treatment of tuberculosis and the knowledge of the immune response gained from targeted antigen delivery to the lungs.

**Grant:** 1R01HL068019-01A1  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** MOLLER, DAVID R MD  
**Title:** Etiologic Antigens in Sarcoidosis  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/06/01-2007/05/31

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology that involves the lungs in over 90 percent of affected individuals and may cause end-stage fibrosis, cor pulmonale, and death. The pathologic hallmark of sarcoidosis is non-caseating granulomatous inflammation. Since extracts of diseased tissue injected intradermally elicit a nidus of granulomatous inflammation in patients with sarcoidosis that is indistinguishable from spontaneously arising granulomas (the Kveim reaction), we postulate that sarcoid tissue extracts contain disease-relevant antigens. Biophysical properties of the active component in Kveim extracts include relative heat stability, resistance to neutral detergents and proteases, and a dependence on tertiary structure. The overall goal of this application is to identify these pathogenic tissue antigens in sarcoidosis. Our central hypothesis is that sarcoidosis is caused by linked T and B cell immune responses to aggregates of altered proteins of microbial origin. Consistent with this hypothesis, our preliminary studies demonstrate the presence of a small number of protease-resistant, neutral-detergent insoluble proteins that by immunoblot analysis are targets of T cell dependent IgG from patients with sarcoidosis but not healthy controls. MALDI-TOF mass spectrometry and immunoblot analysis has identified the mycobacterial catalase-peroxidase protein from *Mycobacterium tuberculosis* (mKatG) or *M. smegmatis* in these protein fractions from sarcoidosis but not control tissues. Preliminary studies demonstrate both T and B cell responses to mKatG proteins in sarcoidosis, suggesting the mKatG proteins are relevant, pathogenic antigens in sarcoidosis. To test the hypothesis that mycobacterial KatG proteins are pathogenic antigens in sarcoidosis, we propose studies to determine the presence of mycobacterial KatG proteins in sarcoidosis and control tissues using MALDI-TOF mass spectrometry and protein immunoblot analyses. To determine whether these microbial proteins induce disease-specific immune responses, we will determine the molecular basis of the B and T cell immune responses to both *M. tuberculosis* and *M. smegmatis* KatG proteins and selected peptides, and determine whether mKatG proteins preferentially expand specific V $\alpha$ /V $\beta$  expressing T cells in patients with sarcoidosis and control subjects. Together, these studies offer the potential of identifying a specific group of microbial antigens involved in the pathogenesis of granulomatous inflammation in sarcoidosis, thus providing a novel target for therapy of this disease.



**Grant:** 1R01HL068518-01  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** KERNODLE, DOUGLAS S  
**Title:** Antisense Mutants of M. Tuberculosis  
**Institution:** VANDERBILT UNIVERSITY NASHVILLE, TN  
**Project Period:** 2002/08/01-2005/06/30

DESCRIPTION (provided by applicant) Advances in mycobacterial genetics have made it feasible to alter virulent M. tuberculosis strains to create novel vaccine candidates. We have constructed mutants of the virulent M. tuberculosis strain H37Rv exhibiting diminished production of iron-manganese superoxide dismutase (SOD). These strains are tolerated better than BCG by mice and when administered as a vaccine, confer greater protection than BCG against subsequent infection with virulent M. tuberculosis, virtually eliminating the lung destruction that is characteristic of pulmonary tuberculosis. The focus of this application is to elucidate the mechanism of superior vaccine efficacy of our SOD-diminished H37Rv strains compared to BCG. We hypothesize that two major factors are involved. The first is the production of key antigens by H37Rv that BCG lacks. The second is a difference in the mechanism of antigen presentation, most likely apoptosis-associated MHC Class I antigen processing, that enhances the efficacy of the SOD-diminished H37Rv strain by stimulating a stronger immune response to antigens common to both BCG and H37Rv. The focus of this work involves elucidating the relative importance of these factors, with specific objectives that include: (1) To determine the direct effect of diminishing SOD production upon vaccine efficacy and immune correlates of protection; (2) To assess the importance of species and strain differences upon the vaccine efficacy of SOD-diminished M. tuberculosis complex strains including the amount of cross-protection generated against other M. tuberculosis complex strains; and (3) To determine whether modifying SOD-diminished BCG and SODdiminished M. bovis by adding specific antigens that are unique to M. tuberculosis improves vaccine efficacy against virulent M. tuberculosis. The findings from this work will help to optimize vaccine efficacy and may also expand our diagnostic capacity to distinguish prior vaccination from tuberculosis infection.

**Grant:** 1R01HL068874-01  
**Program Director:** TOLUNAY, ESER  
**Principal Investigator:** SUMMERSGILL, JAMES T PHD  
**Title:** Proteomic Analysis of Persistent Chlamydia pneumoniae  
**Institution:** UNIVERSITY OF LOUISVILLE LOUISVILLE, KY  
**Project Period:** 2001/12/01-2004/11/30

DESCRIPTION (provided by the applicant): A significant amount of data, including sero-epidemiological and direct detection studies, have suggested a potential role for chronic Chlamydia pneumoniae infection in human atherosclerosis; however, a direct causal role remains to be established. Elucidation of potential pathogenic mechanisms is critical to establishing whether C. pneumoniae is merely an "innocent bystander" in atherosclerosis or possesses biological features consistent with a significant role in the initiation or exacerbation of this disease. The complete genome of C. pneumoniae has been recently sequenced and this information is important for the comprehensive investigation of proteins which C. pneumoniae can produce under various growth conditions, a process known as proteome analysis. Proteome analysis can be important for elucidation of potential functions of expressed proteins, since changes in the bacterial proteome depend on growth stages, disease states or environmental conditions. Atherosclerosis is a chronic inflammatory disease featuring an increased accumulation of smooth muscle cells, macrophages, foam cells, T-cells and lipids within the arterial intima in response to injury. Evidence exists that localizes C. pneumoniae within foam cells of human atheromas. In order for C. pneumoniae to play a causative role in atherogenesis, it would need to persist within intimal tissue for extended periods of time, thereby stimulating a chronic inflammatory response. An alteration of the normal growth cycle of C. pneumoniae can be induced, in vitro, by cytokine pretreatment of the host cell, leading to the induction of a "persistent" form of the organism. Persistence of C. pneumoniae in cells of the developing atheroma, a microenvironment containing a multitude of host cytokines, could induce a differential expression of specific bacterial proteins. Such proteins could serve as virulence factors or immunogens of C. pneumoniae, or as targets for therapeutic intervention or vaccine candidates. Investigation of C. pneumoniae protein expression in persistently infected cells by proteomic analysis will identify bacterial proteins with potential functions in atherogenesis, thus strengthening the case for a causal role of this organism in atherosclerosis. Hypothesis: Growth of C. pneumoniae in the presence of cytokines induces an altered (persistent) form that correlates with a shift in the bacterial proteome. This hypothesis will be tested by (1) determining, by proteomic analysis, alteration in protein expression patterns of C. pneumoniae when induced into an altered (persistent) growth cycle by cytokine treatment; (2) determining the role of IDO activation in the IFN-g-mediated shift in protein expression patterns of C. pneumoniae when induced into an altered (persistent) growth cycle; and (3) determining role of additional cytokines in the shift in protein expression of C. pneumoniae when induced into an altered (persistent) growth cycle.

**Grant:** 1R01HL068912-01A1  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** BARBIERI, JOSEPH T  
**Title:** Molecular pathogenesis of *P. aeruginosa* in CF  
**Institution:** MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI  
**Project Period:** 2002/07/15-2006/06/30

DESCRIPTION (provided by applicant): The morbidity and mortality for most cystic fibrosis (CF) patients is caused by *Pseudomonas aeruginosa*, however its pathogenesis during early stages of infection of children with CF is poorly understood. *P. aeruginosa* possesses several virulence factors, which contribute to its pathogenesis. A relatively new class of virulence factor is the type III cytotoxins, which are delivered into eukaryotic cells upon the direct binding of the bacterium to the eukaryotic cell membrane. These cytotoxins subvert the host innate immune system, allowing establishment of initial foci of infection. This proposal represents a consortium among researchers at the Medical College of Wisconsin, the National Institutes of Health (NHBLI and NCI) and the Wisconsin CF Neonatal Screening Project and will provide insight into the pathogenesis of *P. aeruginosa* in children with CF. Recent studies have shown that components of the type-III apparatus are commonly expressed in adult CF patients who are infected by *P. aeruginosa* and that sera from children with CF contain antibodies against the type-III apparatus and the type III cytotoxin, ExoS. Two hypotheses will be tested: (i) stable colonization of the lung of children with CF requires expression of the type-III system of *P. aeruginosa* and (ii) the immune response to antigens of the type-III system is an early and accurate measurement of infection of the lung of children with CF by *P. aeruginosa*. This proposal will conduct retrospective and prospective longitudinal studies to determine the expression of the type-III system of *P. aeruginosa* in children with CF and to correlate this data with stable infection by *P. aeruginosa*, clinical outcome, the child's genotype for host modifier genes. There are three specific aims: measure the immune response to the type-III system of *P. aeruginosa* in children with CF; identify type-III antigens and immunogens of *P. aeruginosa* isolated from children with CF, and characterize the biological and biochemical properties of type-III cytotoxins from *P. aeruginosa* isolates from children with CF and antibodies to type-III cytotoxins. Completion of these studies will define the molecular properties of *P. aeruginosa* during early infections of children with CF and develop strategies to accurately detect *P. aeruginosa* infections, as well as, provide insight towards the identification of vaccine candidates to delay or prevent infection.

**Grant:** 1R01HL069050-01  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** PARK, PYONG W PHD  
**Title:** Proteoglycans in Microbial Pathogenesis and Host Defense  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2001/12/01-2005/11/30

DESCRIPTION (provided by applicant) Microbial infection is a major public health threat that can be associated with high mortality, and that can also often amplify and lead to chronic inflammation, also resulting in serious complications. The current emergence of multi-drug resistant strains adds to the threat of infections. These features are especially evident in compromised patients in whom drug-resistant microbial pathogens infect with high mortality and morbidity. During infection, microbes exploit a variety of host components to promote their pathogenesis. Among these, cell surface heparan sulfate proteoglycans (HSPGs) are targeted by a wide spectrum of microbes. Cell surface HSPGs function as selective regulators of various molecular interactions, including those important to microbial pathogenesis and host defense. These HSPGs not only function at the cell surface, but also in the extracellular environment as soluble HSPGs because they can be shed as intact ectodomains in response to tissue injury, including those caused by infections. The long term objective of this research is to delineate how cell surface HSPGs regulate, in part, the highly complex host response to microbial infections. This proposal focuses on the role of syndecan-1, the predominant cell surface HSPG of epithelia. The goal of this application is to elucidate the molecular mechanisms that are responsible for exploitation of syndecan-1 shedding by bacterial pathogens to enhance their lung virulence. Three inter-related hypotheses will be tested in three aims: Specific Aim 1. Binding of certain virulence factors to their host receptors triggers signaling events that lead to activation of syndecan-1 shedding will be assessed by determining in molecular detail how LasA, a virulence factor for *Pseudomonas aeruginosa* lung infection, activates syndecan-1 shedding; Specific Aim 2. Syndecan-1 ectodomains regulate the host response by inhibiting innate defense mechanisms will be evaluated by establishing whether syndecan-1 ectodomains, via their specific structural features in their HS chains, inhibit the activity of cytokines and antimicrobials to enhance bacterial virulence in the lung; and Specific Aim 3. This mechanism is used by several major pulmonary bacterial pathogens will be probed by evaluating whether *Staphylococcus aureus* exploits syndecan-1 shedding to enhance its lung virulence. These studies, which delineate how cell surface HSPGs such as syndecan-1 are exploited by microbes for their pathogenesis, should provide a foundation for the development of novel prophylactic and therapeutic agents to combat infections caused by major opportunistic bacterial pathogens.

**Grant:** 1R01HL070146-01  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** PATEL, RAKESH P PHD  
**Title:** Role of S-nitrosohemoglobin in Sepsis  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL  
BIRMINGHAM  
**Project Period:** 2002/04/01-2006/03/31

DESCRIPTION (provided by applicant): Septic shock is a leading cause of mortality in hospitalized patients and is defined as the systemic inflammatory response to infection. This disease is characterized by hypotension and inflammatory damage to organs including the lung and which ultimately leads to multiple organ failure and death. Nitric oxide (NO) has a critical role in this disease with NO produced from iNOS receiving much attention. However, the role of NO in sepsis remains unclear with both detrimental and beneficial roles reported. An important regulator of NO function in the vasculature is Hemoglobin (Hb). Recent insights into the reactions between NO and Hb have shown that in addition to reactions with the heme, NO also interacts with a critical cysteine residue forming S-nitrosohemoglobin (SNOHb). SNOHb can elicit NO dependent effects and has been investigated principally in the context of physiological regulation of blood flow. The potential role of SNOHb in pathology has not been considered however. Recent studies show that SNOHb is elevated during endotoxemia and in this proposal, the novel concept that SNOHb mediates endotoxin induced hypotensive and inflammatory responses, by modulating production of different redox congeners of NO is put forward. The biological role of redox derivatives of NO, including nitroxyl anion (NO<sup>-</sup>) in disease remains largely unexplored although functions in promoting vasodilatation and stimulation of inflammatory responses have been suggested. Preliminary data presented herein suggest that SNOHb dependent vasorelaxation occurs via formation of NO<sup>-</sup> and vasorelaxing effects of red blood cells purified from endotoxin treated rats are demonstrated. These observations have led to the hypothesis that systemic hypotension and inflammation observed in sepsis are mediated by SNOHb. This hypothesis will be tested by pursuit of the following specific aims: 1) Determine the mechanism of SNOHb formation in endotoxic shock; 2) Determine the vasodilatory mechanisms of SNOHb; and 3) Investigate the role of SNOHb as a mediator of inflammatory damage in the lung. Accomplishment of these aims will yield novel insights into both the molecular mechanisms by which NO impacts upon the pathogenesis of septicemia and on possible therapeutic strategies to treat this inflammatory disease.

**Grant:** 1R01HL070147-01  
**Program Director:** LINK, REBECCA  
**Principal Investigator:** SOMMER, STEVE S PHD  
**Title:** Translational Bypass in Patients with Hemophilia  
**Institution:** CITY OF HOPE NATIONAL MEDICAL DUARTE, CA  
CENTER  
**Project Period:** 2002/09/15-2004/08/31

DESCRIPTION (provided by applicant): We hypothesize that small molecules that readily enter cells can induce nonsense suppression by the protein synthetic apparatus such, that nonsense mutations are translationally bypassed at levels up to 20 percent. Evaluation of efficacy will be performed with the prototype drug gentamicin, an aminoglycoside antibiotic. If successful, translational bypass therapy could be beneficial for a significant minority of patients with severe genetic disease. Hemophilia is chosen as the model disease. Major effects of severe hemophilia A)B can be eliminated with only a slight increase in factor level. The hemophilias are an advantageous system to determine directly the efficacy of gentamicin gene therapy because many patients with nonsense mutations are available, the protein product can be measured readily and the kinetics of accumulation and decay can be determined over a short period since the proteins turn over rapidly. The proposed study has four specific aims: 1.Assess gentamicin suppression of nonsense mutations in an initial set of ten patients with severe hemophilia B. 2.Determine if there is a correlation between gentamicin-induced nonsense suppression and gene (factor VIII or IX), stop codon type, and sequence context. 3.Determine whether gentamicin suppresses frameshift mutations in five patients with hemophilia A or B and missense mutations in five patients with hemophilia A or B. 4.Determine whether the effect of gentamicin can be maintained with regular administration of gentamicin for up to twelve weeks. Nonsense suppressors could revolutionize therapy for hemophilia in underdeveloped countries where factor replacement is not readily available and carries risks of blood-borne pathogens. An efficacious nonsense suppressor should be effective in nonsense mutations in any of the 30,000 -40,000 human genes. Nonsense suppressors also may be beneficial in the treatment of cancers that result from nonsense mutation in tumor suppressor genes.

**Grant:** 1R01HL070212-01  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** SAID, SAMI I  
**Title:** Modulation of Lung Inflammation and Cell Death by VIP  
**Institution:** STATE UNIVERSITY NEW YORK STONY BROOK, NY  
BROOK  
**Project Period:** 2002/04/01-2007/03/31

DESCRIPTION (provided by applicant): The broad, long-term objective is to achieve a deeper understanding of the pathogenesis of acute lung injury (ALI) causing the Acute Respiratory Distress Syndrome (ARDS), and to develop more effective means of its treatment and prevention. Hypotheses to be tested: 1) ALI due to oxidant stress is attributable in large measure to two fundamental and interrelates processes: inflammation and cell death by apoptosis or necrosis; 2) Excitotoxic mechanisms are a major pathogenic factor in cell death due to oxidant stress; and 3) Vasoactive intestinal peptide (VIP) and the related pituitary adenylate cyclase activating peptide (PACAP) can effectively reduce or prevent at least some forms of ALI, by blocking these critical processes, and by promoting cell survival pathways. Specific Objectives fall into two broad categories: a) Establish the role of inflammatory, apoptotic and excitotoxic mechanisms in the pathogenesis of ALI. This goal is justified by the need to establish criteria for evaluating the second objective; b) Determine the efficacy, potency, and specificity of VIP & PACAP in the protection against ALI, with special reference to the above pathogenic mechanisms, and the receptors and pathways mediating this protection. 1) Document the importance of key inflammatory mechanisms in ALI, including NF- KB activation, an the expression and production of major pro- and anti-inflammatory cytokines. NF-KB activation, will be assessed by evaluating evidence for such activation (nuclear displacement of p50 and p65 subunits) in models of ALI, and correlating the activation with the degree of injury, and the inhibition of activation with protection from injury. Transgenic mice with targeted deletion of NF-KB subunit p50 will be examined for possible resistance to injury. 2) Assess the contribution of apoptotic and necrotic cell death to ALI by: morphologic evidence, TUNEL-testing, caspase activation, poly (ADP-ribose) polymerase PARP) activation (which often leads to necrosis) or its degradation (a marker of apoptosis), and the degree of protection afforded by anti-apoptotic measures, including caspase inhibitors and upregulation of anti-apoptosis protein bcl2, relative to pro-apoptotic BAX. 3) Validate the concept that endogenous glutamate receptor activation is an important mechanism of oxidant-induced ALI. 4) Determine the mechanisms, receptors, and pathways of lung protection by VIP and PACAP in these experimental models of injury: a) Excitotoxic lung injury due to excessive glutamate receptors due to excessive activation of glutamate receptors, a model that we recently characterized and is uniquely suited to a critical analysis of cell death mechanisms in lung injury; b) oxidant injury due to paraquat and xanthine + xanthine oxidase; and c) endotoxin lipopolysaccharide (LPS)-and Fas- induced apoptotic cell death. Injury will be induced in isolated lungs, cell preparations (alveolar macrophages, alveolar epithelial & pulmonary endothelial cells), and in vivo model of endotoxemia. Whenever available, we will study selected strains of transgenic mice either lacking or overexpressing key factors regulating the inflammatory response, death pathways and survival signals. In searching for the pathways of injury and its

modulation by VIP/PACAP, we will use the newly introduced gene microarrays.



**Grant:** 1R01HL070297-01  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** MARTIN, THOMAS R MD  
**Title:** Molecular Mechanisms of Lung Inflammation  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/04/01-2002/07/31

DESCRIPTION (provided by applicant): The sepsis syndrome is often a systemic consequence of localized infections in the lungs. The mechanisms that initiate and modulate inflammatory responses in the lungs of humans with sepsis need to be better defined in order to design specific therapies that can be used to protect the lungs and systemic organs. Bacteria and their products in the lungs and systemic circulation initiate the sepsis syndrome, in part through specific recognition molecules on the surface of leukocytes and other cells in tissue and the circulation. The major goal of our ongoing studies is to understand how innate immune mechanisms initiated via specific pattern recognition receptors on the cell surface initiate and perpetuate acute lung injury and sepsis syndrome. Our Specific Aims are: 1) to define the pathways that mediate host responses to bacterial products in the lungs of normal humans and patients with ARDS; 2) to define the cells in the lungs that express the major pattern recognition receptors for gram negative and gram positive bacterial products, and the changes in expression that occur in acute bacterial pneumonia; 3) to determine the role of pattern recognition receptors on leukocytes and non-myeloid cells (CD14, TLR2, TLR4 and the signaling protein MyD88) in the clearance of gram positive and gram negative bacteria from the lungs, using mice with targeted gene deletions; 4) to determine whether blockade of CD14, TLR4, TLR2 and/or MD2 protects rabbits from the deleterious systemic effects of localized lung infections. The results of these continuing studies will provide important new information about the mechanisms that control the response to bacterial products in the lungs, and the consequences of inhibiting specific pattern recognition pathways in the lungs and the systemic circulation.

**Grant:** 1R01HL070321-01  
**Program Director:** CROXTON, THOMAS  
**Principal Investigator:** SHAPIRO, STEVEN D MD OTHER AREAS  
**Title:** Macrophage Elastase in Host Defense  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2002/04/01-2006/03/31

DESCRIPTION (provided by applicant): Matrix metalloproteinases (MMPs) are a group of matrix degrading enzymes whose aberrant or excessive expression can lead to a variety of tissue destructive diseases. Less is known about the normal physiologic functions of MMPs. We present data that macrophage elastase (MMP-12) is the only MMP that has direct antimicrobial activity. MMP-12 acts within the lung macrophage as the first line of defense against microbes within the alveolar space. MMPs are well known for their roles in promoting tumor progression. However, with the discovery of angiostatin, an antiangiogenic proteolytic fragment of plasminogen, it became clear that proteinases can be involved in limiting tumor growth. We present evidence that MMP-12 plays a major role in limiting tumor growth within the lung. This property might have clinical importance since at least 6 phase 3 trials using MMP inhibitors for cancer therapy and two for arthritis were stopped last year related to this under-appreciated property of certain MMPs to limit tumor growth. To further define the role of macrophages and MMP-12 in host defense against bacteria and tumors in the lung, we propose to: 1. Test the hypothesis that MMP-12 represents a novel macrophage-mediated intracellular antimicrobial agent. We provide preliminary data that MMP-12<sup>-/-</sup> mice have a poorer outcome in response to *S. aureus* pneumonia, MMP-12<sup>-/-</sup> macrophages have impaired intracellular killing of *S. aureus*, and show that MMP-12 has direct antimicrobial capacity. This activity is independent of catalytic capacity and involves the non-catalytic C-terminal domain. Studies are proposed to define the spectrum of bacteria influenced by MMP-12. We will also define the structural components of MMP-12 responsible for this activity. 2. We will extend the hypothesis that MMP-12 interferes with tumor growth via inhibition of angiogenesis and further define potential mechanisms of action. We provide preliminary data that MMP-12 is required to maintain dormancy of Lewis lung cell carcinoma (LLC) metastases. This activity appears related to inhibition of angiogenesis. This is not merely due to generation of angiostatin. Additional antiangiogenic protein fragments play a role, and we postulate that MMP-12 also interferes with MMP-2-mediated promotion of tumor growth. MMP-12 might do this by cleavage of MMP-2 as well as by competition with MMP-2 for endothelial cell and tumor cell binding through its C-terminal domain. 3. We will determine the role of macrophages in lung development, bacterial infection, and tumor progression. We will take advantage of MMP-12 macrophage specific expression and complete generation of diphtheria toxin (DT) "knock-in" to the MMP-12 locus. We hypothesize that this will result in mice deficient in lung (and peritoneal) macrophages, and that these mice will undergo normal lung development. If this hypothesis is correct, then the mice will be used to study the requirement of macrophages in host defense and inflammation. If the mutation is lethal or not fully deficient in pulmonary macrophages, then lung-specific transgenic mice will be used to inducibly express DT in lungs of mature mice.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R01HL070641-01  
**Program Director:** COLOBINI-HATCH, SANDRA  
**Principal Investigator:** WIZEL, BENJAMIN PHD  
**Title:** CD8+T cell responses to Chlamydia pneumoniae  
**Institution:** UNIVERSITY OF TEXAS HLTH CTR AT TYLER, TX  
TYLER  
**Project Period:** 2002/07/15-2007/05/31

DESCRIPTION (provided by applicant): Chlamydia pneumoniae is an intracellular bacterium that causes a spectrum of respiratory infections and is associated with cardiovascular atherosclerotic disease. Although most studies on C. pneumoniae have focused on providing evidence for its pathogenic role in atherosclerosis, knowledge of the chlamydial antigens and immune mechanisms that lead to protective or adverse immune responses remains limited. Because CD8+ T cells play a critical role in defense against most intracellular pathogens, we hypothesize that C. pneumoniae infection in mice primes a multispecific CD8+ CTL response capable of inhibiting chlamydial growth and that CTL epitope-based immunization strategies can reduce bacterial burdens in the lungs of challenged mice. We will test this hypothesis through the following aims: 1) Identify C. pneumoniae CD8+ CTL target antigens and define the mechanisms by which CD8+ T cells inhibit bacterial growth. We will identify antigens of the murine CD8+ CTL response to C. pneumoniae using MHC class I binding motif-bearing synthetic peptides from putative chlamydial CTL target antigens and mass spectrometric analyses of MHC class I-bound peptides from bacteria-infected cells. Lung and splenic epitope-specific CD8+ CTL will be tested for their capacity to inhibit development of chlamydial inclusions in infected cells through secretion of type 1 cytokines and lytic /apoptotic molecules. We will also use real-time PCR and immunohistochemistry to detect effector molecules in lung tissue. Effector mechanisms will be evaluated in vivo by assessing bacterial loads in lungs of C. pneumoniae-challenged mice after adoptive transfer of CD8+ CTL lines genetically deficient of effector individual molecules. 2) Characterize the response kinetics of C. pneumoniae epitope-specific CD8+ CTL during infection. We will study primary and recall CD8+ T cell responses in the lungs and spleens of C. pneumoniae-infected mice by ELISPOT, confocal microscopy, and flow cytometry with activation and memory cell markers, and MHC class I tetramers. 3) Determine the capacity of CD8+ CTL-based immunotherapies to prevent or ameliorate C. pneumoniae infection. Selected CTL epitopes will be used to generate peptide- and DNA-based vaccines that will be tested for their capacity to reduce bacterial burden in the lungs of C. pneumoniae-challenged mice. These studies will enhance our understanding of the role of CD8+ T cells in protection against C. pneumoniae and will suggest methods of immunological intervention in infections caused by C. pneumoniae and other chlamydial pathogens.

**Grant:** 1R01HL070876-01  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** COLE, ALEXANDER M PHD  
**Title:** Proteomics of Staphylococcus aureus nasal carriage  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2002/04/01-2004/03/31

DESCRIPTION (provided by applicant): The human nasal mucosa is an accessible and clinically important model for the study of microbial interactions with host defenses. Nasal carriage of Staphylococcus aureus (SA) is the most common clinical disorder of mucosal host defense but its molecular and cellular basis is not understood. The disorder is of increasing clinical importance because nosocomial infections are commonly spread by nasal carriers of methicillin-resistant SA and other SA strains increasingly resistant to antibiotics. Our evidence indicates that colonization may be due to impaired innate antimicrobial activity of nasal fluid. The current proposal aims to identify the underlying cause(s) of SA carriage by examining protein determinants of SA carrier airways colonization and comparing microbicidal components of SA carrier fluid to nasal fluids from donors that are not colonized with SA. We hypothesize that 1) noncarriers express antimicrobial (poly)peptide factors some of which are either lacking or defective in SA carrier fluid, 2) altered or deficient host defense factors in airways fluid contribute to the progressive colonization of SA in carriers, and 3) correcting the dysregulated components will restore the antimicrobial activity of SA carrier airway fluid against isolates of SA. To test these hypotheses, we will: 1) detect cationic (poly)peptides that are differentially expressed between nasal fluid from donors carrying SA and fluid from donors that are not colonized with SA utilizing a novel two-dimensional gel electrophoresis (2-DE) approach, 2) identify (poly)peptides that are differentially expressed between donors carrying SA and donors that are not colonized with SA, and 3) reconstitute the antimicrobial activity of SA carrier nasal fluid by replacing abnormal or missing (poly)peptides with intact counterparts purified from noncarrier fluid. Our proposed studies represent a novel approach to identify and link human airway disease biomarkers (cationic polypeptide antimicrobials) with their effects (SA nasal carriage). Together, these studies will for the first time characterize the host resistance factors for a common and increasingly important source of nosocomial infection, and will develop a neglected but very useful natural model for the study of the interactions of bacteria with an accessible mucosal surface.

**Grant:** 1R01HL071113-01  
**Program Director:** BERBERICH, MARY ANNE  
**Principal Investigator:** VISCARDI, ROSE M  
**Title:** Determinants of Ureaplasma-mediated neonatal lung injury  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD  
**Project Period:** 2002/07/01-2004/06/30

DESCRIPTION (provided by applicant): Respiratory tract colonization with Ureaplasma urealyticum (Uu) in preterm infants is a highly significant risk factor for Bronchopulmonary Dysplasia (BPD). In a prospective cohort of infants less than 32 wk gestation, 50 percent moderate-severe BPD, 29 percent mild BPD, and 19 percent non-BPD subjects were Uu tracheal aspirate (TA) positive. Five of six (83 percent) infants who were TA Uu positive and serum or CSF PCR positive had BPD, suggesting invasiveness of the organism may be associated with increased pulmonary pathogenicity. However, invasive disease alone was not significantly associated with BPD, suggesting local immunomodulatory effects of Uu in the lung are required to develop BPD. We previously observed that Uu respiratory tract colonization in preterm infants was associated with greater levels of the pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin (IL)-1B in TA during the first week of life. In vitro studies of cultured preterm monocytes, Uu serovar 3 alone stimulated TNF and IL-8, and, in combination with bacterial endotoxin (LPS) augmented LPS-induced TNF release, but blocked LPS-induced IL-6 release. We used a clinical Uu isolate (biovar 2) to develop the first non-neonatal mouse model of Uu pneumonia. While these mice develop little detectable illness and minimal signs of lung injury, Uu infection caused a biphasic influx of neutrophils peaking 24h and again 14d after inoculation, and of macrophages peaking 48h and 14-28d after inoculation. Our long-term objective is to determine how Uu modulates the pulmonary immune response alone and in the presence of co-inflammatory stimuli. The specific aims of this proposal focus on the central hypothesis that the Uu serovars; differ in their ability to promote the development of BPD due to differences in capacity to augment the pulmonary inflammatory response. The specific aims are: 1) determine whether the risk for BPD is Uu biovar or serovar specific by PCR typing of archived TA isolates, blood and CSF samples from preterm infants with and without BPD, and 2) characterize pathologic properties of TA Uu isolates from BPD and non-BPD infants using our cell culture and murine Uu pneumonia models. These studies will provide new insights into how certain serovars contribute to the prolonged inflammatory process in BPD.

**Grant:** 1R01HL071233-01  
**Program Director:** TOLUNAY, ESER  
**Principal Investigator:** AGRAWAL, ALOK PHD  
**Title:** Structure-Function Relationships of C-Reactive Protein  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 2002/08/01-2002/10/31

DESCRIPTION (provided by applicant): C-reactive protein (CRP) is a major human acute phase protein and a component of the innate immune response. Its serum concentration is increased during inflammatory states, persists for the duration of the inflammatory process and returns to its normal low concentration following subsidence of inflammation. While CRP is felt to play a significant role in inflammation and host defense, the mechanisms by which CRP exerts its effects are unclear. In vitro, CRP binds to phosphocholine (PCh) moieties and can then bind to complement C1q and activate the classical complement pathway. In addition, binding of CRP to phagocytic cells via Fc receptors, with a variety of functional consequences, has been described. Recent publication of the crystal structures of CRP has provided insight into the amino acids that mediate binding of CRP to PCh, to Fc receptors, and to C1q, permitting generation of CRP mutants incapable of binding to PCh and to Fc receptors, as well as incapable of activating complement. Most known functional activities of CRP, in vitro, are associated with ligand-binding and subsequent complement activation or phagocytosis. Accordingly, we will employ such mutants to define the roles of binding to PCh and Fc receptors, and of complement activation in 2 model systems: a) the protective role of CRP in bacterial infections and b) the putative role of CRP in the pathogenesis of atherosclerosis resulting from its ability to bind to enzymatically-degraded LDL (E-LDL). Our specific aims are: 1. To precisely define the ligand-binding sites on CRP required for binding to PCh, FcR and C1q and to generate mutants lacking these critical binding capabilities. 2. To define the role of these 3 binding capabilities in the protective effects of CRP in infection with *Streptococcus pneumoniae*, known to bind to CRP, and *Salmonella typhimurium*, which does not. We hypothesize that both complement activation and phagocytosis will be found to be involved in CRP-mediated protection of mice from bacterial infections. 3) To define the role of the 3 binding capabilities of interest on CRP-E-LDL interaction, and the role of such interaction in the pathogenesis of atherosclerosis. Our working hypothesis is that all 3 binding sites participate in the pathogenesis of atherosclerosis, by binding to E-LDL and initiating complement activation and uptake of E-LDL by macrophages. We will also determine the effects of injecting wild-type and mutant CRPs on the size of the atherosclerotic lesions formed in ApoE knock-out mice. These studies will provide substantial insight into the mechanisms by which this ancient protein may contribute to host defense, or alternatively, to pathogenesis of disease.

**Grant:** 1R01HL071241-01  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** CHAN, JOHN R. MD  
**Title:** Cell Migration in Tuberculosis Infection  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2002/08/01-2007/05/31

DESCRIPTION (provided by applicant): The granuloma plays an important role in host defense against *M. tuberculosis*. The mechanisms that regulate the formation and maintenance of the tuberculous granuloma are, however, poorly understood. Chemokines and chemokine receptors play an essential role in cell migration in both physiological and pathophysiological states. Emerging evidence suggests a role for chemokine and chemokine receptors in regulating the granulomatous response during infection. *M. tuberculosis* has the ability to modulate chemokine and chemokine receptor expression in both in vitro and in vivo systems. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is essential for the control of tuberculosis, and is a potent regulator of chemokine expression and leukocyte trafficking. We have shown that neutralizing TNF- $\alpha$  in mice with persistent tuberculosis results in disease recrudescence associated with granuloma disorganization and diffuse cellular infiltration in the lungs. Based on these observations, we propose to test the hypotheses that: i) chemokines and chemokine receptors play an important role in orchestrating cell migration and granuloma formation in tuberculosis; and ii) TNF- $\alpha$  regulates the granulomatous response by directing the trafficking of immune cells at the site of infection via regulation of specific chemokines and chemokine receptors. Because of the importance of Type 1 T cells in host defense against *M. tuberculosis*, efforts will be focused on examining a subset of chemokines and receptors that can modulate migration of these T lymphocytes. Murine tuberculosis models, as well as immunohistochemical, laser microdissection, and realtime PCR techniques will be used to characterize the expression of these specific chemokines and receptors during tuberculous infection. Mice with disruption of specific chemokine receptor genes and ligand neutralizing reagents will be exploited to dissect specific chemokine network. Similar techniques, in conjunction with in vitro cell migration assays and the TNF- $\alpha$  neutralization model of murine reactivation tuberculosis, will be employed to evaluate the effects of TNF- $\alpha$  on the expression of specific chemokines and receptors, as well as on migration of T cells and monocytes during tuberculous infection. These studies should yield valuable information that will shed light on the roles of chemokines and receptors on cell migration, granuloma formation, and host defense in tuberculous infection.



**Grant:** 1R01HL071554-01  
**Program Director:** PEA VY, HANNAH H  
**Principal Investigator:** BISHAI, WILLIAM R MD  
**Title:** LATENCY AND REACTIVATION TUBERCULOSIS  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2002/06/01-2007/04/30

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis is remarkable in its ability to infect the human host and remain quiescent for many years only to reactivate when host defenses are suppressed. One-third of the global population is latently infected with tuberculosis, yet this clinically inactive state when bacilli are often non- cultivatable is poorly understood and inadequately characterized. Appropriate animal models that more accurately mimic human diseases are needed to test vaccine candidates, and to understand the complex relationship between mycobacteria and host. The currently available animal models in mice and guinea pigs are characterized by multibacillary disease and are distinguished by the host response. Mice mount a poor delayed type hypersensitivity response and develop chronic lung disease, eventually succumbing to a progressive granulomatous pulmonary disease with high bacillary load. Guinea pigs also have a multibacillary disease, but are exquisitely susceptible with rapid hematogenous dissemination and a strong DTH response that results in rapid lung inflammation, destruction and death. Paucibacillary latent disease can only be induced with the administration of antibiotics. In contrast, rabbits are relatively resistant to aerosol infection with M. tuberculosis and mount a granulomatous response that effectively contains the bacilli. Over the course of 6-12 months, all culturable bacilli disappear. In addition, the histopathologic response is remarkably similar to that of humans pointing to the rabbit model of tuberculosis as a promising avenue by which to study stage-specific changes in both host and bacilli. In this application, we will aerosol infect rabbits and allow the granulomatous lung lesions to regress to latency. With the use of immunosuppressive agents such as corticosteroids, iNOS inhibitors, and specific anti-cytokine antibody, we will reactivate infection. In parallel, we will refine the in vitro granuloma assay using rabbit white blood cells to have an in vitro model with which to correlate and compare our in vivo results. Harvesting serum and rabbit tissue at various stages of infection, we will characterize the stage-specific host humoral and cell-mediated immune responses. Understanding the antibody expression profile during latent disease may lead to important diagnostics in a disease hampered by diagnostics with low sensitivity and specificity. In addition, we will analyze the transcriptional bacterial response to various disease stages using microarrays and RT-PCR with molecular beacons. Finally, we will use a transposon mutant library to identify clones that are impaired in specific stages of infection. Appropriate animal models are critical to the successful development of tuberculosis vaccines, new drugs and better diagnostic tests for tuberculosis.

**Grant:** 1R01HL071730-01  
**Program Director:** TOLUNAY, ESER  
**Principal Investigator:** STEPHENS, RICHARD S PHD  
**Title:** Cellular Microbiology of Chlamydia pneumoniae  
**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA  
**Project Period:** 2002/09/30-2006/08/31

DESCRIPTION (provided by applicant): Atherosclerosis has been associated with infection by the bacterial pathogen Chlamydia pneumoniae. The broad, long-term goal of the proposed studies is to define the mechanisms of C. pneumoniae pathogenesis that promote coronary artery disease. The research focus of this application is the molecular characterization of C. pneumoniae interactions with mammalian host cells during acute and persistent infection. The hypothesis is that C. pneumoniae produce components that elicit responses by the host cell and it is the consequent host cell responses including proinflammatory chemokines, growth factors and coagulation factors that mediate pathogenesis and arterial disease. The important role of persistent infection, characteristic of chlamydial infections, on cellular responses and staging the focal environment for chronic inflammation and consequent tissue damage will be evaluated. The significance of these studies is the identification of the cellular mediators of pathogenesis and the chlamydial products that elicit these responses thereby informing new approaches for control and diagnosis by identifying novel targets for diagnostic detection and vaccine or chemical intervention. The specific aims will be investigated utilizing oligonucleotide and DNA array formats to measure changes in transcription in parallel for approximately 30,000 human genes and 1,100 C. pneumoniae genes. Human cellular responses will be tested in cells known to be infected by chlamydia in diseased arterial tissues including vascular endothelial cells, smooth muscle cells and macrophages. The specific aims are: 1) Determine the spectrum of cellular responses to infection by C. pneumoniae. 2) Validate the protein expression of C. pneumoniae-induced changes in host cell gene transcription. 3) Characterize gene expression profiles for C. pneumoniae during persistent infection and during growth in different cell types. 4) Identify chlamydial products and test persistently-expressed C. pneumoniae gene products for their ability to induce host cell responses.

**Grant:** 1R01HL071735-01  
**Program Director:** TOLUNAY, ESER  
**Principal Investigator:** BYRNE, GERALD I PHD  
**Title:** Vascular Molecular Pathogenesis of Chlamydia pneumoniae  
**Institution:** UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN  
CTR  
**Project Period:** 2002/09/30-2006/08/31

DESCRIPTION (provided by the applicant): Chlamydia pneumoniae infection is associated with atherosclerosis progression, destabilization of atherosclerotic lesions, and cardiovascular disease yet our understanding of how this organism may causally contribute to chronic diseases is not well understood. C. pneumoniae is an obligate intracellular prokaryotic bacterial pathogen that can modulate its interaction with host cells to produce either acute productive infection or chronic persistent infection. Isolates of C. pneumoniae exhibit a finite number of genomic polymorphisms suggesting that intra-strain heterogeneity reflect variants suited for growth or persistence in different in vivo environments or host cell types. We propose to compare respiratory and cardiac C. pneumoniae isolates, plaque, clone and analyze individual variants by polynucleotide polymorphism analysis to identify different genotypes that may be present within single strains or selected in different in vivo environments. We will then test these genotypes in models of in vitro infection to identify strains with propensities to cause chronic infection and atherosclerotic disease. Molecular genetic analysis of the growth of these genotypes using microarray gene expression profiling and quantitative real time polymerase chain reaction will identify subsets of genes that are predictive of acute and chronic diseases. These objectives will be accomplished according to two specific aims involving (1) plaquing, cloning and analyzing chlamydial variants obtained from respiratory and cardiovascular sites and, (2) molecular analysis of pathogen and host cell gene usage patterns during differing growth conditions. These investigations will provide useful information on the degree of genetic heterogeneity inherent in C. pneumoniae populations and how this heterogeneity may contribute to the pathogenesis of C. pneumoniae disease. Results also will have the practical application of providing information to obtain the identity of subsets of C. pneumoniae genes or their products that can be used to target the development of new diagnostic tools or intervention strategies to populations that are at risk of heart disease with a C. pneumoniae-mediated component and to the identification of chronic chlamydial infections in general.

**Grant:** 1R01HL072718-01  
**Program Director:** PEAUVY, HANNAH H  
**Principal Investigator:** NATHAN, CARL F MD INTERNAL  
MED:IMMUNOLOGY  
**Title:** TARGETING DEFENSES OF MYCOBACTERIUM TUBERCULOSIS  
**Institution:** WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY  
UNIV  
**Project Period:** 2002/09/30-2005/07/31

DESCRIPTION (provided by applicant): *M. tuberculosis* (Mtb), a leading cause of death in people with HIV/AIDS and a bioterrorism threat, persists in macrophages, where reactive nitrogen intermediates (RNI) from inducible nitric oxide synthase (iNOS) and reactive oxygen intermediates (ROI) from phagocyte oxidase are opposed by Mtb's RNI/ROI resistance mechanisms. RNI kill in part via intrabacterial conversion into peroxynitrite (OONO-) (PN). We have discovered a biochemically novel PN reductase/peroxidase (PNRP) in Mtb, comprised of 4 proteins: AhpC, AhpD, succinyl-coenzyme A acyltransferase (SucB) and lipoamide dehydrogenase (Lpd). Lpd uses its flavin to transfer electrons from NADH to the lipoamide cofactor covalently coupled to SucB. SucB's lipoamide transfers electrons to a Trx-like active site we have identified by X-ray crystallography within a novel fold in AhpD. AhpD's Cys130 and Cys133 cooperate to transfer electrons to the disulfide in oxidized AhpC. The AhpC disulfide arises after formation of a sulfenic acid intermediate during reduction of PN to nitrite or peroxides to the alcohol. Besides participating in this antioxidant pathway, Lpd and SucB appear to serve as shared, perhaps essential components of all of Mtb's alpha-keto acid dehydrogenase complexes (pyruvate, alpha-ketoglutarate and branched chain ketoacid dehydrogenases), thereby supplying much of the acetyl coenzyme A arising from endogenous sources. Acetyl CoA is essential for the glyoxylate shunt that sustains bacillary persistence and for fatty acid synthesis required by Mtb to build its cell wall. Thus, Mtb Lpd and SucB stand at a crossroads between antioxidant defense and intermediary metabolism. In the work proposed here, we will knock out Lpd, SucB and AhpD and study the impact on growth of Mtb in vitro; its sensitivity to RNI, ROI and control stresses; its growth in macrophages that are wild type, deficient in iNOS, deficient in phagocyte oxidase or deficient in both enzymes; and its growth in these four strains of mice. We will solve the crystal structures of Lpd and SucB and identify chemical inhibitors of the three enzymes by screening chemical libraries and by directed synthesis. This work will characterize Lpd, SucB and AhpD as potential targets for interventions that may cripple the growth of Mtb while sensitizing the organism to RNI, ROI and chemotherapy for improved prophylaxis and treatment in normal and immunocompromised hosts.

**Grant:** 1R01HL072871-01  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** MATALON, SADIS PHD PHYSIOLOGY  
**Title:** Modulation of innate immunity in lung transplantation  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL  
BIRMINGHAM  
**Project Period:** 2002/09/30-2006/07/31

DESCRIPTION (provided by applicant): A multi-center clinical trial sponsored by Fujisawa Healthcare, Inc, was planned to compare the efficacy of treating lung transplant patients with tacrolimus and sirolimus versus tacrolimus and azathioprine in reducing the incidence of acute rejection during the first twelve months after lung transplantation. Infection is a secondary endpoint and is assessed throughout the trial (i.e. for 3 years after randomization). Presently the mechanisms by which these agents may modify lung innate immunity have not been identified. Herein, we are proposing to isolate SP-A and AMs from the bronchoalveolar lavage fluid (BALF) of patients participating in this clinical trial to identify differences in the ability of AMs to kill gram positive and gram-negative bacterial pathogens and to identify differences in quantity of SP-A and modifications thereof. These data will be correlated with incidences of infection and rejection in patients participating in the clinical trial. We are also proposing to identify basic mechanisms by which normal but not nitrated SP-A enhances phagocytosis. These goals will be accomplished by completing the set of measurements outlined in the following specific aims: (1) Measure levels of surfactant lipids and SP-A in bronchoalveolar lavage (BAL) samples from patients treated with tacrolimus and sirolimus vs. tacrolimus and azathioprine. Oxidative modification to SP-A (oxidation and nitration) will be assessed by Western blotting, ELISA and mass spectrometry analysis using techniques already established in our laboratory; (2) Quantitate levels of inflammatory cytokines (TNF $\alpha$ , INF $\gamma$ , IL-6 and IL-1 $\beta$ ), as well as levels of nitrate and nitrite, the stable end products of NO metabolism, and nitrotyrosine in the BAL of these patients; (3) Assess the extent of pathogen killing (*Klebsiella pneumoniae*, a gram negative bacterium and *Staphylococcus aureus*, a gram positive bacterium) by AMs isolated from the lungs of these patients in the presence of SP-A and surfactant lipids, and (4) Identify putative mechanisms responsible for decreased ability of oxidized or nitrated SP-A to mediate pathogen killing by AMs. We propose that SP-A binding to AM receptors leads to activation of phospholipase C (PLC) which releases 1,4,5 inositol triphosphate (IP3) resulting in Ca<sup>2+</sup> release from the endoplasmic reticulum. SP-A nitration may lead to decreased binding, diminishing or abrogating intracellular Ca<sup>2+</sup> changes. Our data may provide mechanistic insight into why some patients may develop clinical infection and acute and chronic rejection and thereby form the rationale basis for choosing between these two immunosuppressive regimens for the management of patients with lung transplantation.

**Grant:** 1R21HL072177-01  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** FLORES-VILLANUEVA, PEDRO O MD  
**Title:** MCP-1 and susceptibility to active tuberculosis  
**Institution:** DANA-FARBER CANCER INSTITUTE BOSTON, MA  
**Project Period:** 2002/08/10-2004/05/31

DESCRIPTION (provided by applicant): Our long-term objective is to develop new approaches for the treatment of tuberculosis (TB) by studying the host genetic factors controlling the development of effective cellular immunity against *M. tuberculosis* (Mtb). We have preliminary data showing that the genotype GG in the promoter region of MCP-1 is present in 50% of individuals susceptible to TB. This genotype produces this trait by a mechanism that may involve increased production of MCP-1 and MCP-1 inhibition of IL-12 production through the down-modulation of Osteopontin (Opn) receptor alphaVbeta33 in macrophages. In specific aim 1, we seek to confirm the association of the genotype GG with susceptibility to TB in a two-step case/control and a family study with parental samples of affected individuals and a TDT (Transmission-Disequilibrium Test). The sample for the case/control studies will comprise a large and rigorously characterized group of unrelated individuals with active TB and controls. Plasma samples from these individuals will be used to determine the levels of MCP-1, IL-12, IL-10, IFN-gamma and Opn to uncover the effect of MCP-1 on the levels of these factors. With the same purpose, PBMC from a limited number (20 of each genotype) of these fully typed TB cases and controls will be stimulated in vitro with Mtb antigens and the levels of factors mentioned above measured. In specific aim 2, we will extend our preliminary studies to characterize in vitro the effect of MCP-1 in alphaVbeta33 and CD44 Opn receptors, CD14 and TLR-2, and CD40 and CD154 expression and its relevance in the modulation of IL-12, MCP-1, IL-10, and Opn production in response to Mtb antigens. We also seek to determine if alphaVbeta3/CD44, CD14/TLR-2, and CD40/CD154 have additive effects in the induction of IL-12, and how they interact to modulate each other, and how MCP-1 affects these pathways and these pathways interaction. In specific aim 3, we will explore in vitro how mycobacterial infection affects the production of MCP-1, IL-12, IL-10, and Opn and the expression of alphaVbeta3, CD44, CD14, TLR-2, CD40 and CD154. To achieve our goals in specific aim 1, Dr. Julio Granados, Chairman Investigator, Dept. Immunology, Mexican Institute of Nutrition, will be our consultant and collaborator. He and Dr. Moises Selman, from the Mexican Institute of Respiratory Diseases will recruit the TB cases and controls, process blood samples to isolate DNA, plasma, and PBMC. The PBMC will be stimulated in vitro in Dr. Granados' laboratory. The DNA samples will be sent to Boston where they will be typed. The plasma samples and culture supernatants will be kept frozen until we pick them up (twice a year) and bring them to Boston in vaporize-liquid nitrogen tanks for further analysis. For specific aims 2 and 3, we will use leukophoresis products from fully type- selected individuals of a panel of 200 healthy donors residents in Boston.

**Grant:** 2R01MH050431-05A2  
**Program Director:** WINSKY, LOIS M.  
**Principal Investigator:** LYTE, MARK  
**Title:** Gut to Brain Pathways for Infection-Induced Anxiety  
**Institution:** MINNEAPOLIS MEDICAL RESEARCH FDN, MINNEAPOLIS, MN  
INC.  
**Project Period:** 1994/04/01-2006/06/30

DESCRIPTION (provided by applicant): The experiments proposed in this application examine the effects of infectious microorganisms in the gastrointestinal tract on affect and behavior, effects posited to be mediated by stimulation of a visceral sensory pathway to the central nervous system (CNS). This proposal is based on the demonstration that a low-dose ("subclinical") infection with the food-borne pathogen *Campylobacter jejuni* induces anxiety-like behavior in orally challenged mice. As such, it differs from the current understanding of the mechanisms that govern the ability of infection to influence behavior since these infection-induced behavioral alterations occur in absence of obvious physical sickness symptoms that usually accompany infection-induced behavioral alterations. Further, new data demonstrate that such subclinical infection results in the specific activation of regions within the brain associated with anxiety and that interruption of gut-brain pathways inhibits *C. jejuni* infection-induced brain activation. Thus, our Specific Aims are: 1) To determine the peripheral concomitants involved at the gut level that may be responsible for the ability of subclinical *C. jejuni* infection to induce anxiety-like behavior in mice. The degree of behavioral alterations induced in response to oral challenge with *C. jejuni* will be assessed in conjunction with histochemical and immune analysis of infected animals to extend previous work. We will also examine whether a localized immune activation occurs in the absence of a systemic response; 2) To identify the specific visceral sensory pathways leading from the gut to the CNS that are activated following oral bacterial challenge. We will assess the expression of the activation marker c-fos in enteric and vagal sensory neurons as well as sensory neurons in the spinal chord, and determine the impact of selective hepatic and celiac vagotomy; and 3) To determine connectivity and neurochemical identities of neurons driven by *C. jejuni* infection that form a specific neurocircuitry leading to infection-induced alterations of behavior. Collectively, the above aims will seek to establish that certain behaviors may be modulated, in part, by infectious microorganisms within the gut through the "gut-to-brain" axis involving visceral sensory input. A link between the common occurrence of gastrointestinal infections, especially those which do not produce overt clinical symptoms indicative of infection, with anxiety may therefore identify a previously unidentified circumstance which may play a contributing role in regulating behavior.

**Grant:** 1R01MH063914-01A1  
**Program Director:** THURM, AUDREY E.  
**Principal Investigator:** GOODMAN, WAYNE K MD  
**Title:** Prospective Study of 'PANDAS'  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 2002/04/26-2007/03/31

The existence of PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus) continues to be questioned. Confirmation of a relationship of this putative subtype of obsessive compulsive disorder (OCD) and Tourette's Syndrome (TS) to an antecedent Group A Streptococcal (GAS) has gained considerable practical importance because of implications for treatment and prevention interventions that are radically different from the standard practice of administering psychotropics (such as SRIs or neuroleptics) for symptomatic relief. In particular, a study showing the efficacy of plasmapheresis and intravenous immunoglobulin treatment in PANDAS has stimulated public and professional debate, with some parents demanding these interventions for their children and most clinician-scientists urging caution. The need for further research is clear, but no one study will address all the questions surrounding PANDAS. A series of studies designed to test specific aspects of the pathogen-triggered autoimmune hypothesis will be required. The primary specific aim of the present study is to evaluate whether episodes or exacerbations in obsessive-compulsive (OC) or tic symptoms in children with OCD or TS are significantly associated with antecedent GAS infection, as reflected in elevated serum antibody titers. Seventy-nine children (ages 4 to 12 years) with OCD, TS, or Chronic Multiple Tic Disorder (CMT) will participate in this study and be followed for approximately two and a half years, a 28-month period, at monthly intervals for clinical ratings of neuropsychiatric symptoms and serological testing for GAS infection (ASO and AntiDNase B). The study will be conducted at two institutions, the University of Florida and the National Institute of Mental Health, using identical designs and procedures. In order to identify a cohort that exhibits the desired pattern of exacerbation/regression of symptoms and to allow for attrition, we estimate that approximately 180 patients will have to be enrolled over the five-year study. Blood samples will be obtained at each visit for GAS antibodies and for exploratory immunologic studies. Should clinical signs of pharyngitis develop, throat cultures will be ordered and, if positive for GAS, appropriate antibiotic treatment will be instituted. These cases, estimated at  $n = 45$ , may provide useful information on the effect of antibiotics to attenuate exacerbations or influence OC/tic symptom course. In this cohort with clinical GAS pharyngitis, the rate of exacerbations for the 6-months following treatment will be compared to the corresponding period prior to treatment. The proposed longitudinal study will furnish needed data on the validity of the PANDAS concept and help generate reliable operational criteria for identifying cases at risk of GAS-triggered OC or tic symptoms. The results will help determine if antimicrobial interventions are warranted in certain subtypes of pediatric OCD or TS.



**Grant:** 1R01MH065297-01  
**Program Director:** WINSKY, LOIS M.  
**Principal Investigator:** KIELIAN, TAMMY L PHD MICROBIOLOGY, OTOLARYNGOLOGY  
**Title:** Receptors Involved in Microglial Responses to *S. aureus*  
**Institution:** UNIVERSITY OF ARKANSAS MED SCIS LTL LITTLE ROCK, AR  
ROCK  
**Project Period:** 2002/06/13-2006/05/31

DESCRIPTION (provided by applicant): Microglia are one of the resident mononuclear phagocyte populations within the central nervous system (CNS). These cells share many phenotypical and functional characteristics with macrophages, indicating that microglia participate in innate immune responses in the brain. We have recently demonstrated that microglia are capable of recognizing *S. aureus* and respond by elaborating numerous inflammatory mediators and exhibit bactericidal activity. As such, microglia are uniquely poised to provide an initial line of defense against invading microorganisms into the CNS prior to leukocyte infiltration. However, the receptor(s) responsible for mediating microglial activation in response to *S. aureus* have not been identified. The pattern recognition receptors (PRRs) Toll-like receptor 2 (TLR2) and CD 14 play a pivotal role in macrophage activation in response to the gram-positive cell wall products peptidoglycan (PGN) and lipoteichoic acid (LTA). We have recently revealed that microglia express both of these PRRs which may be responsible for mediating cell activation in response to *S. aureus*. With the goal of defining the role(s) of TLR2 and CD14 in microglial responses to pyrogenic bacteria in the CNS, the following specific aims are proposed: I) To characterize the response of microglia to intact *S. aureus* organisms, PGN, LTA, and secreted virulence factors in terms of inflammatory mediator production; II) To examine the expression and regulation of TLR2 and CD14 on microglia; III) To delineate the functional significance of TLR2 and CD14 expression on microglial activation using microglia from receptor knockout mice, receptor blocking antibodies, and transient transduction of dominant negative receptor constructs; and IV) To examine the importance of microglial TLR2 and CD14 expression in the pathogenesis of *S. aureus*-induced brain abscesses using receptor knockout mice and radiation bone marrow chimeras. Although we are examining microglial activation in response to *S. aureus*, it is likely that our findings will extend to other gram-positive organisms by virtue of their conserved structural components. Understanding the mechanisms by which microglia recognize and respond to microbial products could have a significant impact on a broad range of bacterial infectious diseases in the CNS.

**Grant:** 1R21MH066628-01  
**Program Director:** TUMA, FARRIS K.  
**Principal Investigator:** NORTH, CAROL S MD MEDICINE  
**Title:** Mental Health Consequences of Bioterrorism  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 2002/06/01-2004/05/31

The anthrax attacks subsequent to September 11, 2001 ushered in a new<sup>2</sup> era of bioterrorism threat in the United States. This new area has arrived with no existing systematic database on the mental health effects of bioterrorism to guide our nation's response to it. In developing interventions to address psychological effects of bioterrorism, workers and policymakers can at best only extrapolate from information about other kinds of terrorism and other types of disasters, which may not fit the situation. In the special form of stealth terrorism that makes bioterrorism unique, victims often don't know their exposure status. This disarticulates psychological and behavior response from individuals' perceived exposure to the agent. As a result, mental health risks to individuals from bioterrorism are unpredictable because the risk follows unknown variables other than exposure that are not well understood. Our highly experienced disaster research team proposes to establish new research on the mental health effects of the recent bioterrorism attacks on Capitol Hill. We are uniquely positioned to respond to a narrow window of access to the exposed Capitol Hill population during the annual period of relative legislative quiet, when the affected population is most available to participate in research. This proposed one-year study will start by conducting focus groups with Capitol Hill office staff to identify the relevant issues for study. The information thus obtained will be used to develop interviews about the mental health effects of bioterrorism, to be administered to a random sample of 200 staff workers across Capitol Hill. This research will yield valuable information on mental health responses to bioterrorism and will provide an empirical basis for helping professionals responding to the mental health issues of the affected victims of this and future incidents.

**Grant:** 2R01NS034235-06  
**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:** 1996/09/30-2004/08/31

DESCRIPTION (provided by applicant): The overall goal of our original proposal was to test the hypothesis that concomitant HIV-1 infection impairs clearance of Treponema pallidum from the CSF. The progress that we have made in the first funding period supports our hypothesis. Specifically, individuals with more pronounced HIV-1-mediated immunosuppression are more likely to have neurosyphilis, and normalization of CSF WBC count and serum RPR after treatment for neurosyphilis is slower and less complete in HIV-1-infected individuals. Few studies have addressed the influence of concomitant HIV-1 on CNS infection by T. pallidum. In our study to date, we have enrolled and obtained CSF from 348 subjects with all stages of syphilis. Approximately three-quarters of our subjects are also HIV-1-infected. To date, 53 subjects have had at least one follow-up lumbar puncture. Our ongoing study represents the largest investigation of neurosyphilis in many decades, and is the only study with sufficient power to address the effect of concurrent HIV-1-infection on development of neurosyphilis and the response to neurosyphilis therapy in HIV-1-infected and -uninfected individuals. In this competing renewal application, we focus on three clinically important questions. These questions and the principal hypotheses to be tested for each are: 1) Which HIV-1-infected and -uninfected patients with syphilis should undergo lumbar puncture to evaluate the possibility of neurosyphilis? We hypothesize that individuals with higher concentrations of T. pallidum in blood, those with particular strain types in blood and those with greater diversity of blood T. pallidum strains will be more likely to have neurosyphilis. We will test these hypotheses in Specific Aim 1; 2) How can CSF pleocytosis due to infection with T. pallidum be distinguished from CSF pleocytosis due to HIV-1 infection? We hypothesize that the CSF cellular phenotype and that production of T. pallidum-specific antibody by CSF lymphocytes will distinguish T. pallidum-induced from HIV-1-induced CSF pleocytosis. We will test these hypotheses in Specific Aim 2; and 3) What factors determine response to therapy in HIV-1-infected and -uninfected patients with neurosyphilis? We hypothesize that rapidity and completeness of response to treatment will be related to CSF T. pallidum concentration and to CSF T. pallidum strain type. We will test these hypotheses in Specific Aim 3. The studies proposed in this application are directly relevant to the care of patients with HIV-1 and with syphilis and will ultimately improve our ability to diagnose and treat neurosyphilis.

**Grant:** 2R01RR007861-12A1  
**Program Director:** CHANG, MICHAEL  
**Principal Investigator:** KARP, PETER D  
**Title:** Encyclopedia of E. Coli Genes and Metabolism  
**Institution:** SRI INTERNATIONAL MENLO PARK, CA  
**Project Period:** 1992/08/15-2006/06/30

DESCRIPTION (provided by applicant): SRI International and a group of collaborators propose to further develop the EcoCyc database (DB). The DB is accessible to scientists through the World Wide Web via a user-friendly graphical interface. EcoCyc describes the gene and metabolic pathways to E. coli, and its transporters and transcriptional regulation machinery. It serves as a general reference source for E. coli, and is particularly well suited for use in the analysis of microbial genomes. This project will create new DB modules for several pathogenic E. coli strains whose complete DNA sequence will soon be in the public domain. This addition will facilitate comparative analyses of these strains. EcoCyc is a unique resource for analysis of large gene-expression datasets because it couples an extensive collection of data on transcriptional regulatory mechanisms with a unique program for displaying expression datasets on a full pathway map of the cell. When used as a basis for analyzing newly sequenced microbial genomes, EcoCyc contributes to the identification within those genomes of metabolic pathways that may serve as targets for drug design. Pharmaceutical companies are seeking targets within metabolic pathways as one strategy for developing next-generation drugs against antibiotic-resistant bacteria. EcoCyc is also used for teaching biochemistry by colleges and Universities. SRI further proposes to develop new bioinformatics methods for comparative genomics, and for predicting nutritional requirements and essential genes of an organism from its metabolic network.

**Grant:** 1R01TW005860-01  
**Program Director:** ROSENTHAL, JOSHUA  
**Principal Investigator:** VINETZ, JOSEPH M MD  
**Title:** Leptospirosis Transmission in the Peruvian Amazon  
**Institution:** UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX  
GALVESTON  
**Project Period:** 2002/06/01-2003/06/30

DESCRIPTION (provided by applicant): Leptospirosis, caused by spirochetes of the genus *Leptospira*, is a zoonotic disease transmitted from natural reservoir mammalian hosts to humans. Humans contract leptospirosis through domestic, occupational and recreational activities, which bring them into contact with fresh surface water or wet soil contaminated by the urine of chronically infected mammalian reservoir hosts. It is precisely the ecological relationship between humans and reservoir hosts that leads to leptospirosis in humans. The major hypothesis underlying this project is that the transmission of leptospirosis to humans is substantially increased by both short and long- term anthropogenic influences on the environment. Two specific hypotheses will be tested: 1) Deforestation with resulting changes in land use (land cultivation, fish farming) that bring humans into closer contact with local mammalian fauna and increases in the amounts of surface waters, soil wetness, and changes in soil pH due to short-term weather fluctuations, will lead to an increase in human leptospirosis cases; and 2) Mammalian faunal populations will change due to deforestation and fluctuations in surface waters, including flooding, that will alter the dynamics of leptospirosis transmission. To address these hypotheses, three specific aims will be pursued: 1) Determine the incidence of acute leptospirosis in humans living in diverse biotopes in and near Iquitos, Peru, identify potential places where people are infected, and identify infecting leptospiral isolates to the serovar and molecular levels; 2) Determine the prevalence of leptospiral infection in potential mammalian reservoir hosts, both domestic and wild, in the Iquitos region, map the distribution of infected animals, and identify infecting leptospiral isolates to the serovar and molecular levels; and 3) Delineate the temporal and spatial relationship between anthropogenic environmental changes including deforestation, changes in land use patterns, and changes in mammalian faunal populations on the incidence of human leptospirosis cases. The data obtained from this project will provide insight into the potential of anthropogenic environment changes to alter the transmission of leptospirosis in the Peruvian Amazon. This multi-disciplinary, collaborative project involving Peruvian and U.S. investigators to study a disease of emerging importance in Peru utilizes a diverse set of disciplines (infectious disease epidemiology, mammalian ecology, public health, medical microbiology and molecular epidemiology , and the use of geographic information systems and remote sensing technologies) to answer ecological questions about leptospirosis with widespread general interest to many other fields.

**Grant:** 1R03TW005778-01  
**Program Director:** SINA, BARBARA J  
**Principal Investigator:** KHOSLA, CHAITAN S  
**Title:** Acyl-CoA carboxylases of *Streptomyces coelicolor*: its c\*  
**Institution:** STANFORD UNIVERSITY STANIFORD, CA  
**Project Period:** 2001/12/14-2004/11/30

DESCRIPTION (provided by applicant) Acyl-CoA carboxylases are enzyme complexes with the ability to carboxylate short-chain acyl-CoAs such as acetyl-, propionyl- and butyryl-CoA to yield malonyl-, methylmalonyl- and ethylmalonyl-CoA. These enzyme complexes have been mostly described in actinomycetes, although no information related with their specific physiological role or with their biochemical properties is still available. The broad objective of the present project is the biochemical characterization of two acyl-CoA carboxylases of *Streptomyces coelicolor*. These studies will be mainly directed to identify the source of the substrate specificity/tolerance of these complexes and also to dissect the role of a new characterized subunit in the reaction mechanism of these enzymes. The broad goal of the parent grant is to understand the tolerance and specificity of Polyketide Synthetases (PKSs), which are large multienzyme systems that are responsible for the stepwise biosynthesis of complex natural products from simple 2-, 3-, and 4-carbon building blocks such as acetyl-, propionyl-, butyryl-CoA and their activated derivatives malonyl- methylmalonyl- and ethylmalonyl-Co. The knowledge gained on the biochemistry of the acyl-CoA carboxylases and PKS will be used to try to overexpress both systems in *Escherichia coli* in order to optimize the production of polyketide compounds in this versatile bacterium.

**Grant:** 1R03TW005820-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** HOLMES, KING K MD INTERNAL  
MED:INTERNAL MEDICINE  
OTHER  
**Title:** Randomized Trial/Reduce Recurrence/Bacterial Vaginosis  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2002/01/14-2004/12/31

**DESCRIPTION:** (provided by applicant) Bacterial vaginosis (BV), the most common bacterial vaginal infection in women of reproductive ages, has been linked to female HIV-1 acquisition and infection in both cross sectional and prospective studies. Women in sub-Saharan Africa have the highest prevalence of BV, approximately 40-50 percent in Uganda and Kenya. Thus with such a high prevalence and approximately a 2-fold increased risk of HIV acquisition in women with BV, the attributable risk percent for HIV infection associated with BV is high, and treatment and prevention of prevalent and recurrent BV in African women could potentially have a substantial impact on reducing female HIV-1 acquisition. Although treatment of women with BV using metronidazole successfully eliminates symptoms and signs of vaginal discharge, recurrence rates are high. Clinical trials of treating male partners of women with BV targeting anaerobic bacteria have not reduced the risk of BV recurrence. However, our preliminary work has demonstrated BV-associated morphotypes in the urethra of male partners of women with BV significantly more often than in the urethra of male partners of women without BV. Poor male genital hygiene was also associated with BV in the female partners. We hypothesize that poor genital hygiene in men represents an important risk factor for BV, and that improved male genital hygiene or antisepsis, especially among uncircumcised men, and/or antibiotic treatment of the male partners with broad-spectrum antibiotics (active not only against anaerobes but also against facultative microorganisms) will reduce the frequency of recurrence of BV in women after initial treatment, as compared to antibiotic treatment of the women only. We propose a pilot (phase 2) study to determine the impact of topical antimicrobial/antiseptic use of women undergoing treatment for BV and of their male partners on the risk of recurrence of BV after treatment. We will screen 1000 couples attending two STD clinics in Nairobi, Kenya for BV and other reproductive tract infections, and (to confirm our preliminary studies) to collect smears from the male urethra and subprepuce (from uncircumcised men) to detect BV-associated bacterial morphotypes. Of the 40 percent (N= 400) of women expected to be diagnosed with BV and enrolled in our treatment trial, 380 will have male partners who will agree to be randomized to use of antisepsis or control; and within those 2 arms, the male partners will be evenly randomized to either metronidazole plus azithromycin or placebo. Female participants will be followed at monthly intervals for 3 months, repeating vaginal Gram stains to ascertain cure versus recurrence or persistence of BV. We will also monitor the acceptability and adverse effects with the use of genital topical antisepsis, and occurrence of symptoms or signs of any adverse effects of antiseptic use among the men.

**Grant:** 1R03TW005822-01A1  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** SALGAME, PADMINI PHD  
**Title:** Th1 Cell Apoptosis in Tuberculosis  
**Institution:** TEMPLE UNIVERSITY PHILADELPHIA, PA  
**Project Period:** 2002/08/15-2005/07/31

**DESCRIPTION:** (provided by applicant) The protective immune response operative in tuberculous infection is not completely defined. Nevertheless, existing evidence suggest that cellular Th1-mediated immunity plays a critical role in host defense against Mtb. The protective role of Th1 cell-mediated immune response in TB is perhaps best illustrated by the enhanced susceptibility to Mtb in individuals with human immunodeficiency virus infection, a disease characterized by profound loss of CD4+ T cells, including the Th1 subtype. Recently, work in our laboratory has provided in vitro evidence that Th1 cells selectively undergo CD95-mediated apoptosis. Specifically, CD95-mediated apoptosis in response to CD3/TCR (T cell receptor) complex ligation without engagement of co-stimulatory molecules is observed only in Th1 and not Th2 clones. Therefore, in contrast to Th2 lymphocytes, co-stimulation signals are obligatory to resisting CD95-mediated apoptosis during activation of Th1 cells via engagement of CD3/TCR. Thus activation of Th1 cells by CD3 ligation, a scenario akin to antigen challenge in the absence of co-stimulation, results in apoptosis. Based on these results, we propose to test the hypotheses that i) in tuberculous infection, CD95-mediated Th1 depletion occurs, resulting in attenuation of protective immunity against Mtb, thereby enhancing disease susceptibility; ii) downregulation of the expression of the B7 class of co-stimulatory molecules contributes to Th1 apoptosis. Thus the specific aim of the AIDS-FIRCA proposal is to examine if Th1 apoptosis is occurring in vivo at sites of infection, and if there is a correlation with cytokine production and macrophage costimulation. Specifically pleural fluid and lymph nodes obtained from patients with TB pleuritis and TB lymphadenitis will be investigated. This research will be done primarily at the Tuberculosis Research Centre, Chennai, India.



**Grant:** 1R03TW006001-01  
**Program Director:** SINA, BARBARA J  
**Principal Investigator:** MADDOCK, JANINE R PHD  
**Title:** Characterization of the *Vibrio harveyi* CgtA protein  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2002/07/01-2005/06/30

DESCRIPTION (provided by applicant) GTP-binding proteins play crucial roles in the regulation of fundamental processes in cells of all living organisms. In fact, small monomeric GTP-binding proteins have been found in every organism examined thus far. In eukaryotic cells, these proteins are involved in a number of essential processes, such as signal transduction, protein synthesis and translocation or cell cycle regulation. However, perhaps surprisingly, relatively little information is currently available about roles of GTP-binding proteins in prokaryotes. A subfamily of small GTP-binding proteins, including products of *obg* and *cgtA* genes from *Bacillus subtilis* and *Caulobacter crescentus* respectively, was discovered recently in bacteria. Members of this subfamily are present in diverse organisms ranging from bacteria to humans. On the other hand, functions of these proteins in regulation of cellular processes are largely unknown. Genes coding for these proteins are essential in almost all bacteria investigated thus far. However a viable *Vibrio harveyi* insertional mutant in the *cgtA* gene was described recently by us. Therefore, this mutant gives a unique opportunity to study functions of a member of the subfamily of Obg-like proteins. The goal of this work is to investigate roles of the CgtA protein in the regulation of cellular processes. Our preliminary experiments suggest that the *cgtA* gene product may be involved in regulation of crucial chromosome functions like synchronization of DNA replication initiation, chromosome partition and DNA repair. We plan to perform both genetic and biochemical studies to learn about processes and mechanisms controlled by CgtA and about biochemical functions of this protein. Since there is a strong similarity between CgtA-like proteins in diverse organisms, including humans, we believe that studies planned by us and described in this application are of general biological significance.

**Grant:** 1R03TW006003-01  
**Program Director:** MICHELS, KATHLEEN M  
**Principal Investigator:** BECKWITH, JONATHAN R PHD  
**Title:** Characterization of the bacterial Arc system.  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 2002/07/01-2004/06/30

**DESCRIPTION** (provided by applicant) The Arc two-component signal transduction system of *Escherichia coli* modulates the expression of numerous operons according to the redox condition of growth. This system comprises ArcB as the sensor kinase and ArcA as the response regulator. Under reducing conditions, dimeric ArcB undergoes ATP-dependent autophosphorylation and catalyzes the phosphorylation of ArcA by a His-Asp-His-Asp phosphorelay. Phosphorylated ArcA represses transcription of numerous operons involved in aerobic respiration and activates a small number of operons involved in fermentation. D-lactate, pyruvate, and acetate stimulate ArcB autophosphorylation. Under oxidizing conditions, the accumulating ubiquinone or menaquinone inhibits ArcB autophosphorylation, thereby allowing ArcB to catalyze net dephosphorylation of ArcA-P via an Asp-His-Asp reverse relay. Characterization of the Arc system will deepen our understanding of how bacteria optimize their strategy for energy metabolism. The proposed project has three main aims. The first is to locate the quinone reception site in ArcB and define the mode of action of the signal. The second is to determine which phosphotransfer step in signal transmission and signal decay occurs intermolecularly between the two subunits of ArcB. The last objective is to explore the true role of the cytosolic PAS domain in ArcB, erroneously reported to be the receptor site for the redox signal. This research will be done primarily in Mexico as an extension of NIH grant #R01 GM40993.

**Grant:** 1R03TW006008-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** IRELAND, CHRIS M PHD EARTH/REL  
SCI:OCEANOGRAPHY  
**Title:** HIV and TB Screening of PNG Marine Organisms  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 2002/08/15-2005/07/31

DESCRIPTION (provided by applicant): This research will be performed primarily at the University of Papua New Guinea, in Port Moresby, Papua New Guinea. The objective of this FIRCA application is to develop a bioassay screening program for AIDS and TB at the University of Papua New Guinea to support discovery of new natural product anti-HIV and anti-TB agents from PNG marine organisms. This program represents an extension of a collaboration between Professors Ireland and Barrows at the University of Utah and Professor Matainaho at the University of Papua New Guinea to discover anticancer agents from PNG marine organisms. Dr. Matainaho is currently doing sabbatical research with Drs. Ireland and Barrows at the University of Utah to learn the "CEM-TART" HIV assay. In this system, replication defective HIV-1 is replicated in specially engineered human T-cells. The T-cell lines and virus have been obtained from the NIH AIDS Research and Reference Reagent Program. Dr. Matainaho will transfer this technology back to the University of Papua New Guinea after he returns from sabbatical. In addition, a screening protocol for anti-TB agents will be established based on the Alamar Blue protocol. In preliminary studies, 126 marine invertebrates were collected in the Milne Bay Province of Papua New Guinea. Extracts of the organisms have been prepared and are ready to be screened in the HIV assay. HIV/AIDS has become a serious health concern in Papua New Guinea. Since the report of the first case in 1987, the number of people infected with HIV/AIDS has increased to an alarming proportion with fears of a possible serious AIDS epidemic. In June 2001, the Papua New Guinea National AIDS Council and Department of Health indicated that 3901 people have tested positive for HIV, with about 1366 confirmed with AIDS. The report however, acknowledged that the estimated HIV prevalence to date may be between 10,000 to 15,000. With a weakened immune system, the problem of tuberculosis related to HIV infection is even a more serious threat and potential cause of death. Tuberculosis is listed as a main cause of death in PNG.

**Grant:** 1R21TW006090-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** MASON, PETER R PHD  
**Title:** African Programme for Training in HIV/TB Research  
**Institution:** UNIVERSITY OF ZIMBABWE SALISBURY,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): Infection with HIV has had its most devastating impact on the health of people in sub-Saharan Africa. The burden of HIV and its associated opportunistic infections, particularly TB, is so great that many of the advances in health that have been made are being rapidly eroded. Indicators such as infant mortality and life expectancy show the rapid decline in health status brought about by HIV. In developed countries research has long been recognized as an essential component of development. The resources in developing countries are, however, limited and research is not afforded high priority. Improvements in conditions for research and increased opportunities for researchers to develop their skills is essential for the development of Africa, and this is particularly true in health. This programme will develop both informal and academic frameworks to enable young African scientists to improve their research capacity to a level where they can design, implement and report on meaningful and relevant research. The BRTI has already committed resources to training in health, and has attracted over 300 scientists, technologists and clinicians from 23 countries in Africa to 36 training courses in the past 5 years. The BRTI has also provided a research environment for 21 post-graduate students in Zimbabwe, and has the capacity through interaction in 16 current HIV/TB research topics to attract more. This proposal intends to develop a programme of informal courses that are relevant across a broad range of health research activities. Specific training courses in HIV and TB can be used towards a proposed MSc in either Biomedical Research or Clinical Research. Having achieved these qualifications, support will be given for students to carry out clinical, epidemiological, clinical and laboratory based research. A support framework for clinicians completing M. Med and M.D. programmes will be designed. The BRTI will work closely with local and international collaborators in the development of these programmes.

**Grant:** 1R21TW006096-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** TUPASI, THELMA E MD  
**Title:** Private-Public Collaboration in DOTS & DOTS-Plus for TB  
**Institution:** TROPICAL DISEASE FOUNDATION MAKATI,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): Long-term objectives: 1) to develop the institutional capacity within the MMC DOTS Clinic and the Tropical Disease Foundation to be a national center for training on DOTS-Plus. 2) To develop local expertise necessary for the establishment of treatment centers for the implementation of community-based DOTS-Plus for MDR- TB all over the country, in collaboration with the Department of Health, as a complementary arm to the DOTS program of the National TB Control Service in the spirit of private-public collaboration in TB Control in this high burden country. Short term objective: 1. To develop a competitive application for a Comprehensive ICOHRTA-AIDS/TB program that fosters collaborative and multidisciplinary research on tuberculosis in the Philippines. 2. To strengthen the pilot project on DOTS Plus in order to assess current resources and needs of the institution and to develop a plan for addressing these needs. 3. To identify the staff development and training required in the clinical, laboratory, and program implementation and socio-economic support for a comprehensive training program on the control of tuberculosis including MOR- TB and to identify the resources and capabilities of the partner US-Based institutions in providing these needs. Methods: 1. Staff training in the pilot project on DOTS-Plus at the MMC-DOTS Clinic to gain clinical experience and identify research and training needs to develop and pilot test training modules and to determine the resources required to be met by the proponent institution. 2. Short-term consultations and workshops between and among the key personnel of the TDF and its US-based counterparts 3. Exchange program between the clinic staff and US-based partners including other similar clinics presently collaborating with the to US-based partners, to help in identifying weaknesses and strengths of the current pilot project.

**Grant:** 1R21TW006100-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** KOZLOV, ANDREI P PHD  
**Title:** A New Paradigm for TB/HIVControl in St. Petersburg  
**Institution:** BIOMEDICAL CENTER SAINT PETERSBURG,  
**Project Period:** 2002/09/22-2004/04/15

DESCRIPTION (provided by applicant): The re-emergence of TB, complicated by the emergence of MDR- TB, is one of the greatest public health disasters for modern Russia if not for the entire world. Unlike the TB epidemics in most countries in the catchment ICOHRTA-AIDS/TB Program (=ICATB) areas, the Russian TB epidemic is unique because of: (1) a high prevalence of MDR- TB; (2) a TB epidemic that has not yet been escalated by HIV; (3) a historically effective Soviet-era TB control program that is now highly cost-ineffective and unsustainable in the current fiscal crisis in controlling the new Russian epidemic. This TB control program constitutes both a major part of the present TB problem and a potential part of the solution. The St. Petersburg ICATB Program builds on a strong pre-existing, largely NIH-supported research infrastructure. Following a Planning Grant-enabled period of ensuring political commitment, identification of priorities and suitable partners and creation of a training and research agenda, we will establish a multidisciplinary TB Research Unit at St. Petersburg State University (SPSU) modeled after, juxtaposed to and synergistic with our successful Fogarty AITRP-funded HIV Prevention Unit. Under this model, a multidisciplinary core of scholars from the SPSU Schools of Medicine, Biology, Psychology, Geography and Management and the St. Petersburg civil and prison TB Control Programs will receive long and intermediate term training in the USA and in Russian DOTS and DOTS-Plus pilot projects. Each US trained junior faculty will receive a Re-Entry Grant for TB/HIV-related research upon return to St. Petersburg. Once TB Research Unit staffing complete, we will pursue a research agenda to generate valid, timely evidence-based data to inform. Russian national TE3 decision makers and move towards a new paradigm for Russian TB control. The TB Research Unit in which a multidisciplinary team of well-trained Russian scientists conducts applied research in Russia is likely to influence national decision making in a way that the current "harping" by international agencies has not. The initial research agenda will focus on: (1) a RCT of DOTS vs. standard Russian TB treatment; (2) a DOTS-Plus pilot project following Green Light Committee guidelines; (3) a social network analysis of high TB transmission areas combining methods of molecular biology, epidemiology, medical geography; (4) molecular epidemiology of transmission of TB in prisons.

**Grant:** 1R21TW006101-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** TLOU, SHEILA D PHD  
**Title:** AIDS and TB Research Training Program for Botswana  
**Institution:** UNIVERSITY OF BOTSWANA GABORONE,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): With an HIV prevalence rate of 38.5 percent among adults aged 15 to 49, the most economically productive age group, and a shortage of health care providers equipped to provide AIDS care, Botswana is experiencing an economic and public health crisis. In addition, despite a successful tuberculosis prevention program, particularly regarding the prevention of tuberculosis among those who are HIV positive, tuberculosis is responsible for 20 percent of all hospital admissions and 20 percent of adult deaths in Botswana. While the spread of these diseases offer a challenging future for the country, Botswana is committed to supporting research and interventions aimed at halting these epidemics and caring for those affected. As Botswana undertakes efforts to advance comprehensive responses to the AIDS epidemic- such as providing antiretroviral therapy and establishing new education and outreach programs-developing means by which scientific and operational research capacity increases is critical. Data gathered from such efforts will have important implications for improving care, creating effective prevention methods, and designing better public health programs and policies. Therefore, the development of training programs for those who are to be engaged in clinical, operational, and health services research must be carefully developed so as to build upon Botswana's expertise and to offer new opportunities for those in need of additional information and training. Through this proposal, we will develop a comprehensive research training plan for HIV, AIDS, and tuberculosis in Botswana that meets the specific needs of the country and region, ensures long-term sustainability, and fosters collaboration with other organizations and individuals working within the country .Specific activities for Phase I include community sensitization and education about ongoing and future research efforts, identification of resources for the training program's implementation, and the development of partnerships from a broad spectrum of disciplines and organizations. During the grant year we also will develop a plan to select and recruit people for long-term training at the University of Botswana, Botswana-Harvard Partnership for HIV Research and Education in Gaborone, and at the Harvard AIDS Institute in Boston.

**Grant:** 1R21TW006102-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** SETH, PRADEEP  
**Title:** Planning Grant for ICOHRTA AIDS/TB Program in India  
**Institution:** ALL-INDIA INSTITUTE OF MEDICAL SCIENCES NEW DELHI,  
SCIENCES  
**Project Period:** 2002/09/22-2005/09/21

DESCRIPTION (provided by applicant): Both AIDS and TB are very large health problems in India. The objective of this project is to develop a plan for the coming ten years to strengthen integrated and multidisciplinary research and training at the All India Institute of Medical Sciences (AIIMS), New Delhi, India in the fields of HIV/AIDS and tuberculosis. The University of California in Los Angeles (UCLA) will be the partner institution in the U.S, in this collaboration. Research, training and capacity building needs are truly multidisciplinary and will encompass clinical medicine, drug and vaccine trials, behavioral and social sciences, operational research and health services research. This will include cost-effective strategies for prevention, diagnosis and management of tuberculosis and HIV/AIDS and associated complications, including opportunistic infections. The project envisages training of several categories of health professionals including clinicians, laboratory scientists, research nurses, social scientists, psychologists, biostatisticians, and health care administrators. It will consolidate partnership with other stakeholders in India including Ministry of Health, Directorates of National Programs for Tuberculosis and AIDS, research organizations such as Indian Council of Medical Research (ICMR) and its institutions (Tuberculosis Research Center, Chennai, National AIDS Research Institute, Pune), Department of Biotechnology of the Ministry of Science and Technology, etc. Leadership in key Departments at AIIMS have been identified and agree to participate. Similar steps have been taken at UCLA. Multiple linkages already exist between faculty at AIIMS and UCLA. Administrative support has been obtained at the highest levels. Preliminary meetings took place in New Delhi, February 2002. Key Faculty from AIIMS will visit UCLA, the FIC and other NIH institutes and attend major relevant research meetings in the U.S. between June 2002 and February 2003. Several UCLA Faculty will visit AIIMS. Planning will proceed from June 2002 with repeated opportunities for input and critique by involved faculty at both institutions. This will complement existing or planned U.S. programs including FIC, CIPRA, ICTTRI and international CFAR activities, Current plans for training at UCLA and courses, workshops and cooperative projects at AIIMS in 2002 and 2003 will continue.



**Grant:** 1R21TW006103-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** LAPA E SILVA, JOSE R MD  
**Title:** Innovative approaches for tuberculosis control in Brazil  
**Institution:** FEDERAL UNIVERSITY OF RIO DE JANEIRO RIO DE JANEIRO,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): Tuberculosis is still an important cause of human suffering and economic loss in Brazil and beyond. A concerted effort to address all the questions related to TB control is needed, involving Universities and Health Departments. The Millenium TB Network was organized in 2001 with the goal to generate new knowledge about the disease; to train highly qualified professionals with a critical view of the problem and to transfer the new information to the health system. For many years, leading American academic institutions working in Brazil recognized the importance of this initiative. The major goal of this proposal is to develop a competitive application for a Comprehensive ICOHRTA-AIDS/TB Cooperative Agreement linking Federal University of Rio de Janeiro and other collaborating sites in Brazil and Johns Hopkins University, Cornell University and UC Berkeley as collaborating sites in the U.S. The specific aims are 1) To establish a multilateral collaborative training program in tuberculosis control with investigators and physicians from Brazil and Johns Hopkins, Cornell, and Berkeley Universities. 2) To establish a network system between the different institutions and individuals participating in the program in Brazil and USA in order to promote frequent consultation between the partners to define the type of didactic and laboratory training in health sciences pertinent to tuberculosis control. 3) To identify the needs in infra-structure, human rights protection, administration necessary to the establishment of a long-term collaborative program on TB and AIDS/TB research. The major task of this new consortium will be to identify possible interactions between these partners, in order to guarantee efficiency in the actions. The goal will be to link separate projects, funded by several sources, into one multicenter and organized research and training program to address the TB problem at the national and, in the future, continental levels. During the planning year, projects that share similar objectives will be linked, needs at the collaborating sites, identified, and a common training and research agenda in TB will be developed.

**Grant:** 1R21TW006104-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** BEYERS, NULDA MOTH  
**Title:** A Centre for Tuberculosis Research and Education at the\*  
**Institution:** UNIVERSITY OF STELLENBOSCH TYGERBERG 7505,  
**Project Period:** 2002/09/22-2003/08/21

DESCRIPTION (provided by applicant): Southern Africa has the highest tuberculosis incidence in the world and South Africa is one of the 22 countries in the world that account for 80% of the global TB burden. The Western Cape province of South Africa has one of the highest reported tuberculosis incidences in the world at 500/100,000 population/year. TB, particularly in association with HIV/AIDS, is one of the major problems confronting sub-Saharan Africa where TB is the leading killer of people living with HIV. The Stellenbosch University Faculty of Health Sciences recently chartered a Centre for Tuberculosis Research and Education (CENTRE) with the mission of significantly contributing to the control of tuberculosis through purposeful interdepartmental and multidisciplinary research, education and training in collaboration with other national and international institutions. The CENTRE aims among others are 1. to enhance the collaboration and communication among TB and TB/HIV/AIDS researchers within the University as well as with other research collaborators in the Western Cape, South Africa and southern Africa, and 2. to improve the structured and unstructured education and training of graduate and post-graduate (Masters, Ph.D. and post-doctorate) students and young researchers who investigate adult and pediatric TB and TB/HIV/AIDS in southern Africa. The CENTRE is expected to have a significant impact on the training of researchers with a specific focus of understanding the dynamics of the rapidly spreading HIV/AIDS epidemic in southern Africa and its effect on the TB epidemic. The grant funds are intended to fund the work of a Steering Committee consisting of members from the CENTRE, the Harvard School of Public Health, and other potential co-operating partners. The Steering Committee with the CENTRE Director as Chairman will lead the development of a 2003 Strategic Plan for Graduate & Post-graduate Education in TB/HIV/AIDS Research. By the end of one year the CENTRE Director will prepare and assemble a Phase II Comprehensive Agreement application to fund the subsequent implementation of the 2003 Strategic Plan.

**Grant:** 1R21TW006106-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** GARCIA-GARCIA, MA. DE MD  
**Title:** Mexican Tuberculosis/HIV/AIDS Capacity Program  
**Institution:** MEXICO NATIONAL INSTITUTE OF PUBLIC CUERNAVACA,  
HLTH  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): Tuberculosis (TB), HIV / AIDS and other sexually transmitted infections (STI) are considered public health priorities in Mexico having thus been included in the National Health Plan 2000-20006 of the Mexican Ministry of Health. In Mexico there is a striking lack of formal post-graduate curricula that focus on TB/HIV/AIDS/STI that provide sustainability to building capacity for integrated clinical, operational and health services research. The optimal experience for Mexican trainees is didactic coursework in the context of a structured didactic curriculum and hypothesis-driven mentored research conducted in the context of ongoing projects. The strategy upon which this program will be based is the Creation of a TB/HIV/AIDS/STI Centered Curricula with outreach to other Latin-American and Caribbean countries. During the one year planning grant the specific aims of the present proposal are the following: 1) To solidify collaborative relationships and understandings with all individuals and institutional partners, and assess current resources and needs for developing a comprehensive TB/HIV / AIDS/STI research training plan. 2) To define the type of comprehensive research training programs that will be developed in cooperation with our collaborative partners and in consultation with staff from FIC and other US Government co-sponsors. 3) To develop a step-wise plan, including the identification of training, staff development, and other resources needed to undertake comprehensive TB/HIV / AIDS/STI research training program, and including the development of an organizational structure with links with other relevant national and international organizations conducting and supporting clinical, operational and health services research. 4) To prepare an institutional development plan for the next ten years, demonstrating how resources from a Comprehensive ICOHRTA-AIDS/TB Cooperative Agreement will help the National Institute of Public Health to achieve its long-term development goals related to improved research and training in the areas of HIV / AIDS/STI/TB. The key personnel of this grant have a long standing record of clinical and public health driven international research and training between Mexican National Institutes (the National Institute of Public Health and the National Institute of Medical Sciences and Nutrition) and US academic institutions (Stanford University Medical Center and University of California, San Francisco).

**Grant:** 1R21TW006109-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** SEWANKAMBO, NELSON K MOTH  
**Title:** Enhanced AIDS/TB Care and Prevention Research Training  
**Institution:** MAKERERE UNIVERSITY KAMPALA,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): The Academic Alliance for AIDS Care and Prevention (AAACP), a collaboration of Ugandan and North American Infectious Diseases specialists, was launched in June 2001 with a vision to deliver enhanced HIV care to African patients. A primary objective of the AAACP is to engage in capacity building to train African health care providers (physicians, nurses, pharmacists, counselors, laboratory technologists, and others) in the delivery of advanced HIV care and prevention with the intended goal that these trainees will prioritize knowledge transfer and extend their new knowledge and developed skill sets to regions beyond local AAACP activities. A second equally important objective is to carry out operational research on a large cohort of HIV/AIDS infected patients to learn enhanced strategies including use of ARVs to improve prevention and care of HIV / AIDS in the African setting and use this research infrastructure for training. The resources of Makerere University Faculty of Medicine and the Institute of Public Health will be combined with those of the AAACP into an integrated training strategy.

**Grant:** 1R21TW006111-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** ABDOOL KARIM, SALIM S PHD  
**Title:** Natal-Columbia Clinical AIDS/TB Training Program  
**Institution:** UNIVERSITY OF KWA ZULU-NATAL DURBAN 4041,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): South Africa is currently experiencing one of the worst HIV epidemics in the world and tuberculosis is the most common opportunistic infection associated with advancing HIV disease and AIDS. The recent, substantial increase in numbers of people co-infected with HIV and tuberculosis is exacerbating the existing tuberculosis crisis in South Africa. Building on the longstanding collaborative relationships between the University of Natal and Columbia University with respect to research and training in HIV and tuberculosis through the existing Fogarty AITRP, HVTN and HPTN, we propose to develop a collaborative program in clinical, operational and health services research and training to fill an important training gap in the local response to the HIV and tuberculosis epidemics in South Africa. In order to expand the existing collaboration between the two institutions and the South African Medical Research Council we propose to: i. develop a common vision and understanding of the training program between the US and South African partners; ii. identify the specific research training opportunities and required infectious diseases resources in both the US and South Africa; iii. establish the structure, policies and procedures for the comprehensive ICOHRTA-AIDS/TB co-operative agreement; iv. develop integrated links with other training programs such as the CU-SA Fogarty AITRP; and v. prepare an institutional development plan for the next 10 years demonstrating how ICOHRTA resources will enable the University of Natal to achieve its long-term goals in infectious diseases. The continuum of training concept that has evolved through the CU-SA Fogarty AITRP for HIV and tuberculosis basic science, public health, behavioral and ethics research training where Fellows do coursework in the US and conduct their research in South Africa will be applied to this proposed training program for building clinical, operational and health services research capacity. Training opportunities at Columbia University through the Division of Infectious Diseases in the context of providing clinical care include: consultation rounds for patients on the HIV/TB inpatient care, a unit dedicated to the management of HIV infection and the diagnosis and management of tuberculosis, and augmented by a core conference schedule. The following collaborative projects will provide research opportunities for Fellows in South Africa: HPTN 046, HPTN 035, HPTN 043, HVTN 040, numerous operational tuberculosis projects and new initiatives such as the proposed CIPRA which includes an acute infection and natural history study in a clade C population and strategies to integrate antiretroviral therapy with the tuberculosis directly observed short course therapy program. This planning grant will facilitate the development of an ICOHRTA training program which addresses South Africa's priority needs and the institutional development plan of the University of Natal. It optimally utilizes the Columbia University training resources and strong linkages with existing NIH funded clinical research and other Fogarty training programs through building on a well-established, vibrant collaboration involving Columbia University, the University of Natal, the South African Medical Research Council and potentially the NIH Clinical Center.

Includes Research Project Grants (RPGs)  
Excludes Clinical Trials

**Grant:** 1R21TW006114-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** GOTUZZO, EDUARDO MD  
**Title:** Development ITMAvH-UAB-ITMA Comprehensive ICOHRTA-AIDS  
**Institution:** UNIVERSIDAD PERUANA CAYETANO LIMA,  
HEREDIA  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): The goal of the present ICOHRTA-AIDS/TB proposal to the Fogarty International Center (FIC) is to develop and initiate a pilot tuberculosis (TB)-oriented training program in clinical, health services, and operational research. We intend to use this pilot program as the basis of a competitive comprehensive ICHORTA-AIDS/TB cooperative agreement application for fiscal year 2003. We will ensure that a comprehensive ICOHRTA-AIDS/TB program will be well integrated with other relevant TB, HIV/AIDS, and HTLV-I programs at UPGH and the rest of Peru. The proposed planning grant and subsequent training and research cooperative agreement proposal is a collaborative initiative between three institutions with well-established training experiences in Peru--the Instituto de Medicina Tropical Alexander von Humboldt (IMTAvH) of the Universidad Peruana Cayetano Heredia (UPGH), the University of Alabama at Birmingham (UAB), and the Prince Leopold Institute of Tropical Medicine Antwerp (ITMA)--with the purpose of strengthening the abilities of Peruvian and regional Latin American scientists, physicians, and public health practitioners in sustainable clinical, operational and health services research. The focus of this proposal will be the application of clinical science and health care services research to benefit individuals infected with TB, with an emphasis on those co-infected with HIV and/or HTLV-I.

**Grant:** 1R21TW006117-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** MUGYENYI, PETER N MOTH  
**Title:** AIDS AND TB TRAINING OPPORTUNITIES PROGRAM (ATTOP)  
**Institution:** JOINT CLINICAL RESEARCH CENTER KAMPALA,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): Although the seroprevalence of HIV has declined in Uganda over the past 10 years, the HIV epidemic in Uganda is far from controlled. In the face of the HIV epidemic, tuberculosis rates are high and associated with significant mortality. With the advent of antiretroviral therapy, prevention strategies alone are no longer sufficient to meet the current needs in Uganda. There is now a moral imperative to bring the remarkable advances in the field of HIV to developing countries like Uganda. One key step in the rebuilding of the Ugandan public health infrastructure resulted from a unique collaboration between the Ugandan Ministry of Health, the Ministry of Defense and Makerere University to form the Joint Clinical Research Center (JCRC). The JCRC is a research and health care facility devoted entirely to HIV and leads the way in opening Africa to antiretroviral therapy. Through the years, the JCRC has formed strong collaborations with Case Western Reserve University, the National Tuberculosis and Leprosy Control Programme, and Mbarara University. The proposed training program will build on these strong relationships with the common mission of controlling HIV and TB. The goal of this proposal is to develop a comprehensive training program that will build the Ugandan capacity to translate basic and clinical research findings into public health policy and interventions. The training program will build on a growing number of clinical research projects on HIV and TB and extend the findings of these studies to the public health and policy arena. The specific aims of this application are to form a planning committee, create an institutional development plan, define research and training agendas, and develop plans for program evaluation. This strategy will be developed through a year long series of planning exercises between the investigators at the Joint Clinical Research Center in Kampala, Uganda, Case Western Reserve University, in the US, Mbarara University of Sciences and Technology, National Tuberculosis and Leprosy Control Programme, and Kampala City Council.



**Grant:** 1R21TW006151-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** PAPE, JEAN W MD  
**Title:** Haiti Comprehensive AIDS/TB Research Training  
**Institution:** GHESKIO CENTERS PORT-AU-PRINCE,  
**Project Period:** 2002/09/22-2003/09/21

DESCRIPTION (provided by applicant): This proposal is for the GHESKIO Centers in Port au Prince Haiti to prepare for the ICOHRTA-AIDS/TB program. The GHESKIO Centers, directed by Jean Pape, is a Haitian non-governmental research and training organization working in close partnership with the Haitian Government on HIV and inter-related diseases such as tuberculosis and sexually transmitted infections. Dr Pape and GHESKIO have collaborated with Dr Warren Johnson of Cornell University for 20 years and Dr Peter Wright of Vanderbilt University for 10 years. This long research and training collaboration has culminated in numerous publications and the recent launch of a Phase 2 HIV vaccine trial at GHESKIO. (HVTN Protocol 026) The three partners are currently collaborating on five NIH grants and two UNOP/World Bank/WHO TOR grants for research and training in Haiti. The research base for the ICOHRTA-AIDS/TB program in Haiti will be 1) HIV prevention clinical trials of HIV vaccines and vaginal microbicides through the NIH HVTN and HPTN. 2) Therapeutic clinical trials for adults and children of highly active antiretroviral therapy (HAART) regimens and tuberculosis regimens 3) Operational and health science research in support of an expansion of GHESKIO service activities to 25 departmental health centers in Haiti. This expansion is being supported by the Haitian MOH, the United States Agency for International Development, and Mission of French Cooperation. GHESKIO has a three-step strategy to develop a proposal for The Fogarty International Center's ICOHRTA-AIDS/TB program. The three steps are 1) Consolidate existing collaborations and define the research training program with national, regional, and international partners 2) Establish the GHESKIO organizational structure to accomplish this training program and 3) Prepare a long-term institutional development plan to sustain the program in the future. A series of 4 workshops with key partners will be held that will allow GHESKIO to proceed in a step-by-step fashion towards the development of an ICOHRTA-AIDS/TB proposal.