Research Interests of Training Faculty

Emerging Infectious Diseases Uniformed Services University

2007

Andre, Richard, Ph.D., Professor
Department of Preventive Medicine and Biometrics
Research Description: Malaria and other arthropod-borne diseases have been the emphasis of my research. My studies have dealt with the natural history of these diseases and their arthropod vectors in Asia, Africa, and Latin America. The overall goal of these investigations has been the development of more effective prevention and control measures for U.S. military personnel and other populations at risk.

This research has led to significant scientific contributions in the following three areas: parasite resistance to chemotherapeutic drugs, mosquito biosystematics, and the transmission dynamics of malaria. I participated in studies to delineate, through the use of computerized geographical information systems (GIS) and remote sensing (RS) technology, the macro- and micro-environmental factors that enhance the risk of emerging diseases, i.e., malaria, in susceptible human populations Latin America. This NASA-funded project was in direct support of the Ministry of Health in Belize to focus their malaria control efforts. I have conducted research on human malaria transmission as related to the vector competence of four major vectors in Central and South America. This work was done in collaboration with WRAIR and the Ministries of Health in Belize and Peru. In collaboration with investigators at the US Navy laboratory (NAMRCD) in Peru, I am also studying the vectors (i.e., sand flies) of another human disease, Bartonellosis, to develop a disease risk model of this potentially emerging disease through the use of RS and GIS. Also, I am working on three other grants - one funded by NIH to train public health personnel in Belize, Brazil, and Peru, one funded by NOAA for work on climatic effects on the epidemiology of Bartonellosis in Peru and the third by NIH for work on the effects of human agricultural practices on malaria vectors in Belize.

Broder, Christopher, Ph.D., Professor
Department of Microbiology and Immunology
Research Description: We are pursuing structural and functional analyses on the interactions between enveloped viruses and their cellular receptors through immunological, biochemical, and genetic approaches. HIV-1 and new emerging paramyxovirus agents are the two main areas of research work presently being pursued. The goals of our work are to identify the steps and requirements of viral envelope glycoprotein (Env)-mediated membrane fusion, the determinants of viral tropism, the discovery of new viral receptors, and to dissect the early events which take place during the virus entry process. We are also interested in the structure of these viral envelope glycoproteins with particular emphasis on the immunological characteristics of the native glycoproteins. With the use of recombinant vaccinia virus expressed HIV-1 Env we have carried out an extensive analysis of the antigenic structure of native oligomeric Env, and use of oligomeric Env as a vaccine immunogen, otherwise known as gp140. In addition, in collaboration with other laboratories, we are pursuing novel prime-boost vaccination strategies with particular HIV-1 isolate Env proteins, using Venezuelan Equine Encephalitis (VEE) replicons and soluble oligomeric gp140 immunogen preparations in small animals and non-human primates. The second area of work is relatively new and is the investigation of Hendra virus and Nipah virus, which are newly emerging and highly lethal zoonotic agents. These studies are in collaboration with several scientists located at CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong, Victoria, one of only 4 facilities in the world where zoonotic BSL-4 agents may be researched. Both viruses are new members of the *Paramyxoviridae*, enveloped, negative-sense RNA viruses, and are now the prototypic members of a new Genus, *Henipahvirus*, and are classified as zoonotic BSL-4 agents. They appear to infect through the respiratory system initially and are capable of causing viremia. Hendra and Nipah both have broad species tropism, which is unusual because most paramyxoviruses are species restricted and do not have other reservoirs in nature. Current evidence points to several species of flying foxes (large Australian fruit bats). The potential to be weaponized and used as biological warfare agents is clearly possible. We have developed recombinant gene expression systems to study the attachment and membrane fusion-entry mechanisms of these viruses, and are developing novel reagents that may serve as potential vaccines as well as specifically blocking virus infection and spread.

Burgess, Timothy, MD, MPH;
Director, Viral Diseases Program, Naval Medical Research Unit 2, Jakarta, Indonesia
Research description: We are conducting a number of epidemiologic and basic science investigations of diseases caused by dengue, influenza, chikungunya, Japanese encephalitis, and other viruses endemic and enzootic in Southeast Asia. Dengue viruses are a complex of four closely related but antigenically distinct mosquito-borne, enveloped, single-stranded RNA viruses, which are estimated to cause 100 million infections

annually worldwide. Disease caused by dengue infection ranges from non-specific, self-limited symptomatic febrile illness to self-limited but severe "break-bone fever," to potentially fatal hemorrhagic fever and shock. We are conducting prospective, longitudinal cohort and cluster-based studies of dengue virus transmission and disease, with the objectives of defining the burden of disease and identifying factors associated with disease severity, both acquired and genetic host susceptibility factors as well as viral determinants. In support of these studies, we are developing and evaluating rapid diagnostic assays for dengue infection and for the differentiation of dengue from other febrile illnesses. We are characterizing antibody and cellular responses to dengue infection to examine immunological factors associated disease severity. We are refining a flow-cytometry-based assay for measurement of neutralizing antiviral antibodies. In addition, we are characterizing the viruses currently circulating and causing disease. We have a major effort underway to detect and characterize influenza viruses circulating in Southeast Asia and evaluate immune responses to influenza infection, in order to identify pandemic threats such as the current avian influenza A/H5N1 virus. We are conducting syndrome-based febrile disease etiology studies to identify describe pathogens associated with human disease in specific populations. We are studying the causes of acute respiratory illness and acute meningoencephalitis syndromes, applying multiplex diagnostic assays to define the responsible agents.

Dr. Drusilla Burns
Chief, Laboratory of Respiratory and Special Pathogens, CBER/FDA
Research Description: Secretion systems are critical for pathogenic bacteria since these transporters are responsible for delivery of toxins and effector molecules to target host cells. My laboratory has been studying the type IV family of transporters, members of which are critical for the virulence of *Bordetella pertussis*, *Helicobacter pylori*, *Bartonella* spp., and *Brucella* spp., among others. Type IV transporters are composed of a number of proteins that are believed to assemble to form a transport apparatus that spans the inner and outer membranes of these gram-negative bacteria. While the protein components of the transporters have been identified, a number of important questions remain concerning these transport systems. What is the structure of the transport apparatus? How does the transporter interact with its toxin/effector substrate? How is the toxin/effector transported across the bacterial membrane barriers to gain access to the target host cell? Answers to these questions not only will provide insight into pathogenic mechanisms, but may also yield information that could be used to develop novel therapeutics and vaccines against diseases caused by these organisms. A number of years ago, we discovered the type IV secretion system of *B. pertussis* and demonstrated that pertussis toxin utilizes this system. Since our initial discovery of the Ptl transporter, we have examined the genetic organization of the *ptx-ptl* operon, identified the components of the transporter, determined the form of the toxin that interacts with the transporter, and studied the functions of specific Ptl proteins. We are working to elucidate the series of events that take place during toxin secretion with particular emphasis on understanding the structure of the transporter and the interaction of the toxin with the transporter.

Davies, Stephen, Ph.D., Assistant Professor
Department of Microbiology and Immunology
Research Description: Molecular biology, biochemistry and developmental biology of helminth parasites and the immunobiology of helminth infections. Helminths, or parasitic worms, including nematodes, flukes and tapeworms, collectively infect approximately 2 billion people worldwide, or about a third of the world population. The majority of infected people reside in developing countries in tropical and temperate climate zones, where helminths constitute a significant public health concern, but helminth infections are also of increasing concern to U.S. service personnel, Peace Corps workers and civilians that visit endemic areas.

Blood flukes of the genus *Schistosoma* are second only to malaria as a parasitic cause of morbidity and mortality, infecting approximately 200 million people worldwide and causing potentially life-threatening liver, intestine and urinary system pathology. While there is evidence from animal models and human field studies that host $CD4^+$ T cells can mediate protective immunity against schistosome infection, efficacious vaccines for schistosomiasis have proved difficult to develop. The long-term objective of our studies is to develop new immunotherapies and chemotherapies aimed at inhibiting schistosome development in the definitive human host, thus simultaneously preventing the pathology associated with schistosome infection and blocking parasite transmission. Our studies using a murine model of *Schistosoma mansoni* infection have demonstrated that, paradoxically, schistosomes require signals from host $CD4^+$ T cells to complete their development normally, suggesting that blocking interactions between schistosomes and host T cells might provide a novel approach to interfering with parasite development. Currently we are focused on further understanding how schistosomes activate $CD4^+$ T cells and how T cell responses subsequently inhibit or

facilitate schistosome development. Novel mechanisms by which helminths, as a pose to viruses, bacteria and protists, activate host $CD4^+$ T cells are of particular interest, as is elucidating how schistosomes respond to signals from the host immune system, from signal transduction to gene transcription.

Dey, Saibal, Ph.D. Assistant Professor
Department of Biochemistry and Molecular Biology
Research Description: Human MultidrugTransporter: Mode of Action and Functional Regulation: The effectiveness of anti-microbial and anti-cancer chemotherapy largely depends on the ability of thetherapeutic agents to reach their sites of action. Following administration, the fate of a drug molecule depends on how well it is absorbed from its site of administration, its distribution pattern, the extent and nature of its biotransformation, and on the efficiency by which it is excreted. Even when these obstacles are surpassed, thetherapeutic potency of a drug could be profoundly affected by occurrence of intrinsic as well as acquired drug resistance in the target cells. Thus, strategic development of chemotherapeutic drugs has to continuously battle against poor bioavailability and occurrence of drug resistance. The role of the human multidrug transporter P-glycoprotein (Pgp) in both of these phenomena is rapidly unfolding. Functionally, Pgp is an ATP-dependent efflux pump for an inordinately widerange of structurally unrelated hydrophobic drugs including anti-cancer and anti-HIV agents. In order to retain thetherapeutic effectiveness of chemotherapeutic agents, a major effort is underway to selectively inhibit the function of Pgp in tumor cells as well as in certain normal tissues. Although random screening of natural products and synthetic libraries have shown some promise, a better understanding of the mechanism of Pgp-mediated drug transport is necessary for developing inhibitors with improved efficacy.

Research goals of my laboratory are directed towards 1) elucidation of the molecular mechanism involved in coupling of ATP hydrolysis to drug translocation by Pgp, 2) characterization of its functional regulation by pharmacological agents as well as endogenous molecules and 3) identification of novel therapeutic targets within the protein. We use a vaccinia virus mediated infection transfection protocol for generation of recombinant Pgp molecules and for their rapid biochemical characterization. Baculovirus-mediated expression, in insect cells, allows large-scale production of the protein. Purification and functional reconstitution of Pgp can be achieved by metal-chelate chromatography.

Dubois, Andre, M.D., Ph.D., Professor
Department of Medicine
Research Description: *Helicobacter pylori* adherence in the human gastric mucosa involves specific bacterial adhesins and cognate host receptors. We have shown that alyl-dimeric-Lewis x glycosphingolipid is a receptor for *H. pylori* and that *H. pylori* infection induced formation of sialyl-Lewis x antigens in gastric epithelium in humans and in a Rhesus monkey. The corresponding sialic acid-binding adhesin (SabA) was isolated with the "retagging" method, and the underlying sabA gene (JHP662/HP0725) was identified. The ability of H. pylori strains to adhere to sialylated glycoconjugates expressed during chronic inflammation might thus contribute to virulence and the extraordinary chronicity of *H. pylori* infection. We have also been studying apoptosis in gastric epithelial cells in persons infected with *H. pylori*. Active caspase-3 staining was increased in gastric mucosa from infected persons and animals, compared to uninfected controls by immuno-histochemistry. Stimulation of downstream events leading to apoptosis, such as cleavage of PARP (polyadenosine-diphosphate-ribose polymerase) and DFF45 (DNA fragmentation factor 45) as a result of activation of caspase-3, was evaluated. PARP was cleaved, resulting in the presence of both an 89- and a 24-kDa band along with DFF45, resulting in the presence of 10- and 12-kDa bands in gastric cells exposed to *H. pylori.* Our data show that H. pylori stimulates the activation of caspases and downstream mediators of caspase-induced apoptosis. This suggests that H. pylori -induced apoptosis is mediated through caspase pathways, which include the activation of caspase-8 and subsequent cleavage and activation of caspase-3. This is consistent with caspase-3 activation that was found in the gastric mucosa of humans and monkeys infected with *H. pylori*. We also studied the association between *H. pylori* and gastric cancer, and demonstrated that *H. pylori* can be detected in precancerous lesions and in cancer when using in situ hybridization. *H. pylori* was detected inside metaplastic, dysplastic, and neoplastic epithelial cells, and the expression of the virulence factors *cagA* and *babA2* was co-localized. Importantly, expression of *cagA* was significantly higher in patients with precancerous and cancerous lesions than in controls. These novel findings are compatible with the hypothesis that all stages of gastric carcinogenesis are fostered by persistent intracellular expression of *H. pylori* virulence genes, especially *cagA*, inside precancerous gastric cells and pleomorphic cancer cells. We are presently investigating the role of *H. pylori* in experimental carcinogenesis in Rhesus monkeys.

Geisbert, Thomas W., Ph.D. Adjunct Faculty
Chief, Department of Viral Pathology & Ultrastructure, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)
Research Description: Research opportunities are available for basic or applied research on emerging viral pathogenesis with a particular emphasis on viruses causing hemorrhagic fever including Ebola virus, Marburg virus, and Lassa virus. We primarily carry out studies under Biosafety level 3 and 4 containment to determine the kinetics of host-pathogen interactions using both in vitro and in vivo models. Research focuses on understanding the clinical course and pathogenesis of infection in rodent and nonhuman primate models in order to develop rational and effective countermeasures. We are interested in determining the role of cellular and host immune responses in protection, recovery, and pathogenesis of these diseases. Human vaccines against filoviruses and arenaviruses are being developed and tested.

Giam, Chou-Zen, Ph.D., Professor
Department of Microbiology and Immunology
Research Description: Molecular Biology of human T-lymphotropic virus type I and II (HTLV-I and -II), Kaposi sarcoma-associated herpesvirus/human herpesvirus type 8 (KSHV/HHV-8), hepatitis C virus (HCV). We are particularly interested in how viral regulatory proteins impact on cellular signal transduction and transcription pathways. Efforts on HTLV-I focus on elucidating the mechanisms of action of the HTLV-I transcriptional activator/oncoprotein, Tax, with emphasis on the mechanism via which Tax targets I-κB phosphorylation/degradation, constitutive NF-κB activation, and T-cell transformation. A new area of concentration has evolved based on our recent findings that Tax promotes unscheduled degradation of several key mitotic regulators including cyclin A, Pds1p/securin (anaphase inhibitor) and Clb2p/cyclin B in yeast, rodent, and human cells by activating the Cdc20-associated anaphase promoting complex (APC), APC^{Cdc20}, aberrantly. The Tax-induced dysregulation of APC^{Cdc20} is accompanied by faulty chromosome transmission, severe chromosome aneuploidy, and a loss of cell viability. The mechanism of APC^{Cdc20} activation by Tax and its biological and clinical consequences are being actively pursued. Current studies of KSHV/HHV-8 concentrate on the mechanisms of action of two viral regulatory proteins, Rta and K-bZIP, which control viral reactivation from latency and viral DNA replication. Finally, the study of HCV attempts to address the mechanism of HCV replication and persistence. Cell culture-based methods are being developed to support HCV replication and for examining innate host cell defense mechanisms against HCV.

Grieco, John, Ph.D., Assistant Professor
Department of Preventive Medicine and Biometrics
Research Description: My research efforts focus on the adult and larval ecology of anopheline mosquitoes in the country of Belize, Central America. These studies involve the use of remote sensing and geographic information systems (GIS) and remote sensing (RS) to predict locations of vector breeding sites and to determine the effect of deforestation and agricultural practices on larval habitat and disease distribution. Work also focuses on the vectorial capacity of the main anopheline vectors in Belize to the *Plasmodium* parasite and how this relates to disease transmission. This research effort enables us to better characterize the vectorial role of the *Anopheles* species in the region. Additional research focuses on the behavioral response of insect vectors to chemical stimuli (i.e. attractants and repellents). Laboratory work is centered on a newly developed assay system to evaluate the contact irritant and spatial repellent effects of chemical compounds. This work also involves the testing of chemical compounds in hut studies at field sites in Belize and Thailand. Additional field sites are being planned for Tanzania and Benin. Field work in Belize centers on testing compounds against *Ae. aegypti, An. albimanus, An. vestitipennis* and *An. darlingi*. Studies in Thailand are used to primarily evaluate compounds against *Ae. aegypti*. Future efforts in this are of study will include evaluations of insecticide resistance on behavioral responses. As part of this study will be the mapping of resistant anopheline populations in Belize with an attempt to correlate the agricultural use of insecticides and the behavioral response of target populations. An additional focus of the research will entail determining the mode of action of repellent and irritant compounds in eliciting a behavioral response and the corresponding neurophysiological changes in the insect system. Two other grants also underway are looking at the distribution and control of *Stomoxys* flies in Thailand; and a joint effort with WRIAR for testing topical repellents against *An. albimanus*.

Guerry, Patricia, Ph.D., Professor
Department of Microbiology and Immunology and Enteric Diseases, NMRC
Research Description: Molecular pathogenesis of *Campylobacter jejuni: Campylobacter jejuni* is a major cause of bacterial diarrhea worldwide and the leading cause of food-borne illness in North America. Despite its importance as a human pathogen little is understood about the pathogenesis of *C. jejuni*. Research is focused on characterization of surface antigens of *C. jejuni* and understanding their role in immunogenicity and host cell interactions. Comparative genomic studies have revealed considerable genetic diversity among Campylobacter strains and suggest that there may be important differences among strains in the mechanisms of pathogenesis. The lab is comparing virulence mechanisms among several clinical isolates that behave differently in *in vitro* and *in vivo* models of disease using molecular genetic approaches. These studies include understanding the role of capsular polysaccharide, lipooligosaccharide (LOS) core, and type IV secretion systems in campylobacter virulence. Additionally, campylobacters are unusual among prokaryotes in that numerous proteins are glycosylated, including flagellin. Flagellin is heavily glycosylated with unusual 9-carbon sugars that resemble sialic acid (pseudaminic acid). There is a major research effort aimed at understanding the significance and biological role of flagellin glycosylation. There is an increasing awareness of prokaryotic glycoproteins and campylobacter flagellin affords an excellent model to study the mechanism of glycosylation.

Huggins, John W., Ph.D., Adjunct Faculty.
Chief, Viral Therapeutics Branch, Virology Division, US Army Medical Research Institute of Infectious Diseases (USAMRIID)
Research Description: Antiviral Drug Development focusing of smallpox and monkeypox. The laboratory focuses development of antiviral therapy of viruses of military and bioterrorism concern with primary emphasis on smallpox (variola virus), monkeypox and other pathogenic orthopoxviruses. Historically, smallpox has been used as a biological weapon. Eradication eliminated the disease but did not eliminate the etiological agent, variola virus. Thus, the US Government supports continued research to develop new vaccines, antiviral drugs, and sensitive and specific rapid diagnostic assays, as advised in the Institute of Medicine report, "Assessment of Future Scientific Needs for Live *Variola virus," National Academy Press, 1999, Washington, D.C.*

Research on orthopoxvirus therapeutics encompasses the entire spectrum of drug evaluation from basic drug discovery through development and utilization of animal models for drug evaluation including pathogenesis studies to understand the animal models and to characterize the drug effect. We have developed mouse models utilizing cowpox and vaccinia and non-human primate models of monkeypox and variola utilizing multiple routes of infection and are utilizing those models to evaluate drugs in compliance with the FDA Animal Efficacy Rule utilizing Good Laboratory Practices (GLP). Research involving all viruses except variola is conducted at USAMRIID in our BSL-3 laboratory and work with variola virus is conducted by USAMRIID at CDC, Atlanta in one of only two BSL-4 facilities world wide authorized by the World Health Assembly under World Health Organization oversight to possess and work with variola. Particular emphasis is currently placed on understanding the pathophysiology of smallpox and monkeypox in non-human primates and monkeypox in man. Human monkeypox is a potentially lethal orthopoxvirus infection, clinically resembling smallpox that has reemerged in the Democratic Republic of the Congo (DRC). The Division of Medicine and the Viral Therapeutics Branch are conducting a descriptive study of the clinical, virological and immunologic characteristics of human monkeypox at the Centre Medical Congolais Kole hospital (CMC Kole) in the Sankuru District of Kasai Orientale Province, Democratic Republic of Congo in collaboration with CMC Hospital Kole, *Institut National de Recherché Bio-Medicale* and the Kinshasa School of Public Health. The FDA has indicated that understanding disease pathophysiology will be critical for validation of the primate model of classical smallpox and monkeypox to be utilize under the FDA Animal Efficacy Rule. Toward that goal we will continue studies to better characterize the disease and to evaluate new oral antiviral drug that are active against orthopoxviruses. Our laboratory also supports a ongoing clinical trail of ribavirin treatment of hemorrhagic fever with renal syndrome (HFRS) in Korea, and collaborates with others in the Viral Therapeutics Branch whose primary focus is filoviruses and Disease Assessment Division on evaluation and validation of PCR and immunological assays that are useful tools for our research.

Jerse, Ann, Ph.D., Associate Professor
Department of Microbiology and Immunology
Research Description: Pathogenesis of Neisseria gonorrhoeae: Colonization of the human female genital tract by N. gonorrhoeae can be asymptomatic or cause acute inflammation of the endocervical canal. This pathogen frequently ascends to the upper reproductive tract resulting in scarring of the fallopian tubes and pelvic inflammatory disease. Conventional approaches to studying how N. gonorrhoeae adapts to the female host have been limited by the lack of a small animal model of gonococcal genital tract infection. Pre-clinical testing of vaccines and other prophylactic agents against gonorrhea has also been handicapped by the lack of an animal infection model. To facilitate research in these areas, we developed a murine model of gonococcal genital tract infection in which female mice are treated with estradiol to promote susceptibility to N. gonorrhoeae. Gonococci are recovered from the mouse lower genital tract for at least a week following intravaginal inoculation and an intense inflammatory response occurs in > 50% of mice. We are currently using this model to study how the gonococcus adapts to the microenvironment of the female genital tract by testing the infectivity of mutants in genes that are hypothesized to be involved in evasion of host innate defenses (i.e. complement, phagocytic cells, hydrophobic agents, commensal flora). We are also using this model to identify the host factors that select for, or induce the expression of antigenically variable proteins called opacity (Opa) proteins by N. gonorrhoeae during infection. Finally, development of this model has enabled us to study the effect of various immunization strategies and topically applied vaginal microbicides on the prevention of gonorrhea.

Joseph Mattapallil, B.V.Sc., M.S., Ph.D., Asst. Professor
Department of Microbiology and Immunology
Research Description: Cellular and molecular mechanisms of HIV pathogenesis and anti-viral immunity. Early HIV host interaction severely cripples the immune system by destroying CD4 T cells that are central to the generation of secondary immune responses to previously encountered pathogens and vaccines. This damage appears to be most severe in mucosal tissues (oral, gastrointestinal, rectal and vaginal mucosa) as most of the preexisting memory CD4 T cells reside in these tissues. The extensive loss of these cells very early during infection thereby sets the stage for immunodeficiency and AIDS. Research in my laboratory is focused on four major areas of HIV infection namely, (1) delineating the effect of early HIV infection on the generation and maintenance of long-term anti-viral immune responses (2) identifying the T-cell correlates of protection against HIV (3) delineating the mechanisms of durable mucosal T cell repopulation following anti-retroviral therapy and (4) understanding the role of innate immunity in protection against HIV infection. These studies rely on using powerful cellular and molecular tools such as multi-color flow cytometry, real-time PCR and microarray-based technologies. Due to the difficulties of identifying early HIV infection and mucosal tissue sampling in HIV infected subjects these studies will largely be performed in rhesus monkeys that are experimentally infected with simian immunodeficiency virus, a virus that causes an HIV like disease in monkeys. These studies will provide valuable insights into the mechanisms of HIV pathogenesis and significantly aid in the development of better strategies to control HIV infection.

Kaleeba, Johnan, Ph.D., Asst. Professor
Department of Microbiology and Immunology
Research Description: Mechanisms of infection and molecular pathogenesis of Kaposi's sarcoma-associated herpesvirus (KSHV). Herpesviruses are a diverse family of enveloped viruses that infect human, non-human primate, and other animal hosts in which they can establish life-long infections, often without causing disease. However, in settings of immune incompetence, these viruses can induce opportunistic disease states including encephalitis, blindness, graft rejection or pneumonia in transplant patients, birth defects, mononucleosis, and cancer. My laboratory is interested in the infectious process of KSHV, a new human herpesvirus etiologically linked to Kaposi's sarcoma, body cavity-based lymphomas, and other lymphoproliferative syndromes. Whereas KSHV displays broad target cell tropism in culture, only a limited number of cells may be essential to viral pathogenesis in vivo, reflecting the existence of cell type-specific restrictions to virus infection and replication in vitro and in vivo. We recently identified the widely expressed cystine/glutamate transporter xCT as a receptor for KSHV entry into a variety of cell types (Science 2006, 311:1921). As a direct outgrowth from this discovery we are specifically interested in three conceptually related areas: (a) analysis of KSHV glycoprotein interactions with the host cell surface using fluorescence-based imaging, characterization of viral glycoprotein interactions with the receptor, and delineation of structural attributes that drive formation of molecular intermediates which commit the virus particle to the fusion pathway; (b) determination of the

biological significance of xCT and associated molecules (e.g., integrins) in KSHV infection using biochemical, genetic and cell biological tools including specialized cell lines expressing chimeric “designer” constructs of the receptor and related molecules from other species, (c) identification of cellular processes induced by virion engagement of xCT (including post-entry portals for delivery of the virus genome into the interior of the target cell), coupled with integration of virus-associated signaling to cellular oncogenesis and establishment of the latent state. We are also exploiting the genomic and biologic conservation between KSHV and rhesus monkey rhadinovirus (RRV) as a uniquely accessible natural infection system for studying mechanisms of infection, the evolutionary limits of host receptor usage and tropism, and the pathogenesis of virus-induced disease; we anticipate utilizing this animal model as a basis for rational design of targeted anti-viral agents against infectious disease. Another goal is to examine molecular crosstalk between KSHV infection and signals transduced from inflammatory cytokines that cause upregulation of the KSHV receptor. Related studies using transgenic small-animal models of infection are also being explored to test KSHV dissemination during underlying bacterial or retroviral infections that favor receptor expression via induction of oxidative stress. Additional collaborative efforts with colleagues at the NCI are designed to provide an epidemiological perspective on KSHV tropism by examining the extent to which polymorphisms in the receptor gene can control susceptibility to KSHV infection among distinct populations.

Kochel, Tadeusz PhD, LCDR, MSC, USN, Adjunct Faculty
Director, Virology Program, NMRCD-Lima
My virology program has three main research interests: Arboviruses, Influenza and HIV.
Arbovirus:
• Dengue virus transmission studies in Iquitos, Peru and Maracay, Venezuela. These studies serologically monitor the transmission of dengue viruses in 2500 person cohorts. Additionally, within the Iquitos cohort Aedes aegypti are monitored to determine the mosquito density at which dengue is transmitted.
• Active surveillance for dengue diseases within the Iquitos and Maracay cohorts. Asymptomatic and symptomatic infection rates are determined and correlated with infecting serotype dengue virus and patients serological history.
• Identification of predictors of dengue diseases severity. Cytokine profiles and serotype viral loads are monitored throughout the disease process and correlated with disease severity.
• Febrile surveillance study. The goal of this study is to rapidly identify new and endemic infectious agents that result in acute febrile diseases in South America. Currently, 30 sites are active in Bolivia, Ecuador, Colombia and Peru. Approximately 3,000 specimens are processed per year for pathogen isolation. In addition to bacterial and rickettsial agents, flavi, alpha and arena viruses are identified in this study.
• Primate vaccine trials. NMRCD has an Aotus nancymae colony. The animals are used for dengue virus and alpha virus vaccine candidate efficacy trials.
• Dengue virus vaccine development studies. Vaccination strategies are evaluated in mice prior to advancement to non-human primates.

Influenza:
•Conduct surveillance for respiratory viruses in Central and South America. Currently, 64 sites, in 12 countries, are participating in this study. Approximately, 2,000 specimens are processed, per year, for pathogen isolation.

HIV:
• HIV prevalence and incidence studies in sixteen countries of Central and South America. High risk groups, general population and military are included in these studies.
• Surveillance of HIV genotypes in sixteen countries of Central and South America.
• Determination of the existence and relative importance of circulating recombinant forms (CRFs) of HIV.
• Determination of prevalent risk factors for infection with HIV.

Philip Krause, M.D., Adjunct Faculty
Deputy Director, Division of Viral Products, CBER/FDA
Research Description: 1. Our laboratory is investigating the molecular pathogenesis of herpes simplex virus latency, with an emphasis on HSV-2 and on differences between HSV-1 and HSV-2. We have been studying the latency-associated transcript (LAT) region of the virus, which we have shown controls site-specific reactivation of HSV (the LAT region is the major determinant of whether HSV will reactivate more efficiently at genital or facial sites). Recent studies have focused on the role of these sequences on influencing differences in spread of virus through the nervous system and on molecular mechanisms by which they exert this effect.

2. We are developing highly sensitive but completely non-specific molecular methods that may be used to detect and reveal the sequences of viruses in any biological specimen, without foreknowledge of what virus might be present. We believe that these types of techniques will have great utility in virus discovery, studies of disease pathogenesis, bioterrorism and biological warfare preparedness, and regulatory applications.

Maurelli, Anthony, Ph.D., Professor
Department of Microbiology and Immunology
Research Description: Molecular genetics and regulation of virulence gene expression in Shigella flexneri and in the obligate intracellular pathogen, Chlamydia trachomatis. Shigella are the causative agents of bacillary dysentery while organisms of the genus Chlamydia cause pneumonia, blinding eye infections, and sexually transmitted diseases. Our studies on Shigella flexneri concern identification of secreted virulence products of Shigella, their role in pathogenesis and how these proteins are transported out of the bacteria. Our goal is to apply molecular genetic and structural analyses to determine how the components of the secretion apparatus interact with each other and the virulence proteins in order to promote their passage across the bacterial membranes and out of the cell. A second project involves pathogen evolution and focuses on identifying genes that have been lost from Shigella due to their incompatibility with expression of virulence. Development of molecular tools for genetic analysis of Chlamydia spp.: No genetic tools currently exist for the study of this important human pathogen. We are applying our experience in the study of Shigella to the problem of designing genetic techniques for the study of Chlamydia. Our goal is to elucidate the molecular steps of Chlamydia entry into the host cell and intracellular survival of the pathogen.

Maynard, Ernest, Ph.D., Assistant Professor
Department of Biochemistry and Molecular Biology
Research Description: We are studying novel mechanisms of viral and parasitic infection in order to define molecular targets for disease intervention. We use a wide range of biochemical, biophysical, and genetic approaches in order to study protein interactions that are involved in virus/parasite survival and propagation.
Vif-mediated HIV infection. HIV/AIDS has killed 20 million people, infects 40 million today, and continues to reemerge in multiple drug resistant forms. Virion infectivity factor (Vif) is a virally encoded HIV accessory protein that is essential for the infection of CD4+ T cells. Vif targets host factors and helps to stabilize HIV. For example, APOBEC3G (a host enzyme that helps to destroy viral DNA) is degraded as a result of its interaction with Vif. The Vif-APOBEC3G interaction is therefore a desirable drug target. However, studies of this and other important interactions have been impeded by low solubility and aggregation of Vif. We have developed a method for the purification and refolding of Vif that yields pure, soluble protein. We have identified a novel metal-sensing motif in Vif that is also responsible for its multimerization. An *in vitro* fluorescence assay is being used to study the interaction of Vif with different cellular and viral targets. Such studies will shed light on the the role of Vif in HIV infection and may ultimately lead to the discovery of novel anti-HIV drugs.
Targeting trypanosomes. Compartmentalization of function is a hallmark of eukaryotic cell biology. The glycosome is found in the parasite, *Trypanosoma brucei*, where it encapsulates glycolytic enzymes. Survival of the trypanosome in the host blood stream is dependent on the free energy from glycolysis. In order to understand how glycosomal function is linked to human trypanosomaisis, we are studying the mechanistic details of glycosomal protein targeting. Most glycosomal proteins contain a targeting sequence at their C termini that is recognized by a cytosolic receptor (Pex5). We have developed a sensitive competitive binding assay for measuring the specificity and affinity of Pex5-ligand interactions. We hypothesize that molecules aimed at disrupting glycosomal targeting in typansomes will be lethal.

Merrell, Douglas., Assistant Professor
Department of Microbiology and Immunology
Research Description: Helicobacter pylori and the host pathogen interface: The process of human-bacterial interaction is, more often than not, a complex one that can range from benign symbiotic collaboration to a pathogenic association resulting in death of the host. My lab focuses on the complex interplay that occurs during pathogenic interactions, and how these interactions can lead to the development of disease. Currently, our studies are focused on the gastric pathogen Helicobacter pylori. H. pylori causes gastritis, ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Approximately 20% of those infected with H. pylori ultimately develop some form of overt clinical disease, and it is now accepted that disease outcome is determined by both bacterial and host genetic factors. However, the understanding of the process of disease onset and progression is still in its infancy. Current work in the lab takes a two-pronged approach to investigating the process of H. pylori pathogenesis. First, since H. pylori colonizes and thrives within the human stomach, a site that is inhospitable to virtually all other microorganisms, the bacterium must be able to adapt to the stressful environment. We have taken a genomic approach and used DNA microarrays to define the transcriptional stress response of the bacterium to a number of different microenvironments. These studies are being extended by genetic and biochemical approaches to elucidate the role of individual genes in long-term survival and colonization of the bacterium. Second, we are investigating the host changes brought about by interaction of H. pylori with eukaryotic cells. We have defined host cell transcriptional changes that occur both in vitro (in tissue culture) and in vivo (in the murine gastric tract) upon interaction of the bacterium with host cells. Current studies are further investigating the roles of the effected genes using a biochemical and cell biological approach and attempting to define their expression levels in gastric biopsy samples from patients suffering from gastric cancer.

Metcalf, Eleanor S., Ph.D., Professor
Department of Microbiology and Immunology
Research Description: Host-Parasite Interactions in Salmonella Infections: The long term goals of the research in our laboratory are directed towards an understanding of the molecular and cellular mechanisms which regulate the host responses to Gram negative bacteria and the virulence factors of the bacteria which permit the organism's survival in the host. One aspect of these studies is directed towards understanding Salmonella infections from a bacterial perspective, by delineating the initial steps in the invasion of the host by the Salmonella. Identification of the bacterial and environmental factors which regulate the invasive capacity of Salmonella will permit the development of methods to modulate the host response and thereby increase the resistance of the host to infection. A corresponding project focuses on the intestinal epithelial cells of the host and their potential contribution to the pathogenesis of Salmonella infections. One approach currently underway is to analyze the ability of Salmonella to stimulate these soluble mediators in these cells. The effect(s) of these soluble mediators on other mucosal innate host cells is also under investigation. Understanding the role of these soluble mediators may permit targeting of these molecules as therapeutic interventions. Possible projects include induction of innate immune mechanisms in Salmonella -infected intestinal epithelial cells; interactions of Salmonella -induced products of intestinal epithelial cells with other cells of the innate mucosal immune system; cell surface expression and secretion of Salmonella virulence gene products; identification, characterization and cloning of genes essential for Salmonella virulence.

Mitre, Edward, M.D., Assistant Professor
Department of Microbiology and Immunology
Research Description: Our lab studies the immune response to filariae, tissue-invasive roundworms which are transmitted by insects. Pathogenic human filariae include Wuchereria bancrofti and Brugia malayi, which cause lymphatic filariasis, Onchocerca volvulus, the cause of river blindness, and Loa loa, which causes African eyeworm. Like other helminths, filariae induce a type 2 immune response characterized by eosinophilia, elevated serum levels of Ag-specific and polyclonal IgE, and increases in T-cell production of IL-4, IL-5, and IL-13. Over time, though, chronically infected patients develop a filarial antigen-specific hyporesponsive state, with decreased T-cell proliferation and cytokine production in response to filarial antigen. The mission of our lab is to understand the mechanisms behind the development, maintenance, and cessation of IgE-mediated responses in filarial infections in order to ultimately develop new modalities of prevention and treatment for parasitic, allergic, and autoimmune diseases. To accomplish this, we will be working with both a mouse model of filariasis and with cells from patients infected with filariae. We also have an ongoing

collaboration in which we are trying to determine the underlying mechanism of immune deficiency in patients with the hyper-IgE syndrome.

O'Brien, Alison, Ph.D., Professor and Chair
Department of Microbiology and Immunology
Research Description: Molecular Mechanisms of Bacterial Pathogenesis: One long-term goal of the major research project in the laboratory is to define at the molecular, cellular, and whole animal levels the pathogenic mechanisms by which Shiga toxin-producing *Escherichia coli* (STEC) cause disease. A second objective is to develop strategies for prevention of the potentially life-threatening sequela called the hemolytic uremic syndrome. STEC are food-borne pathogens that cause outbreaks of disease associated with ingestion of undercooked hamburgers or raw milk. Such an outbreak occurred in 1993 in the Pacific Northwest. *E. coli* O157:H7, the prototype STEC, is characterized by the production of Shiga toxins (Stxs) and the capacity to adhere avidly to the large bowel epithelium. Our studies on the virulence mechanisms of STEC include: creation of molecular tools (monoclonal antibodies and DNA probes) for detecting toxin, investigation of the molecular genetics and regulation of toxin synthesis, purification and characterization of toxins, development of small animal models to further clarify pathogenic traits of STEC, evauluation of the molecular mechanisms by which *E. coli* O157:H7 and other STEC, adhere to epithelial cells, and creation of therapies, and vaccines against Stx-producing *E. coli.* Two other on-going projects in the laboratory include: i.) analysis of the molecular mode of action of a newly described Rho-modifying toxin of *E. coli* and its role in the pathogenesis of *E. coli*-mediated urinary tract infections; and, ii.) generation of anti-*Bacillus anthracis* spore monoclonal antibodies as a means of preventing anthrax in individuals who are in danger of exposure or who have been exposed to these spores.

Quinnan, Jr., Gerald, M.D., Professor and Chair
Department of Preventive Medicine and Biometrics
Research Description: Much of our research is directed toward development of a vaccine for prevention of HIV-1 infection. We are attempting to define the basis for the common resistance of HIV-1 strains to neutralization by antibodies, as well as to define how to induce antibodies that neutralize these viruses. These issues are important, because antibodies that neutralize the infectivity of viruses are the usual component of the immune system that determines protection from infection. With success in these studies it may be possible to induce neutralizing antibodies that are effective for prevention. We have made substantial progress toward understanding the basis for neutralization resistance, and the characteristics of an HIV envelope glycoprotein that may be effective in eliciting neutralizing antibodies effective against resistant strains. We have also made substantial progress using an alpha virus replicon system for in vivo expression of HIV envelope protein toward induction of the desired antibody responses in small animals and in monkeys. Another ongoing study is attempting to further improve the system used for immunization of the monkeys. This improved system will be applied for other uses, such as development of vaccines for use in defense against biological threat agents. We have a large grant from NIH which is funding ongoing studies to determine if these responses can protect monkeys from experimental challenge. These studies will indicate whether there would be merit in proceeding to human clinical trials. A recent area of research relates to development of a vaccine to prevent hepatitis C virus infection. This research is focused on developing new approaches to present the envelope glycoproteins of hepatitis C to the immune system for induction of neutralizing antibodies, and the use of alphavirus replicons for delivery of the immunogens.

Richards, Allen, Ph.D., Associate Professor
Department of Preventive Medicine and Biometrics
Research Description: Major research interests are the study of arthropod-borne diseases, especially rickettsial diseases. This not only includes observing their role in nature, but most importantly in utilizing epidemiology, immunology, vaccine and rapid diagnostic assay development to decrease the risk of their detrimental effect on military and civilian personnel. Currently, our department is involved in developing new enzyme-linked immunoassays for scrub typhus, typhus, Rocky Mountain spotted fever, human monocytic ehrlichiosis and human granulocytic ehrlichiosis using recombinant protein antigens. In addition, we have developed several new real-time PCR assays for diagnosis of rickettsial diseases, scrub typhus and louse-borne relapsing fever. Currently, we are developing real-time PCR assays for ehrlichioses and trench fever. Another major focus is the development of a vaccine for scrub typhus utilizing recombinant protein

antigens and DNA constructs. Currently we are able to obtain 80% to100% homologous protection with recently developed trivalent recombinant protein and DNA vaccines in an outbred mouse model.

Schaefer, Brian C., Ph.D., Assistant Professor
Department of Microbiology and Immunology
Research Description: Molecular mechanisms of signal transmission from lymphocyte antigen receptors to NF-kB. We are investigating intracellular signaling events that regulate antigen-dependent lymphocyte activation and proliferation, with particular emphasis on T cell receptor activation of the transcription factor, NF-□B. Our experimental approach involves using live cell imaging and confocal microscopy techniques to study the redistribution of signaling intermediates in the pathway that connects the T cell receptor (TCR) and B cell receptor (BCR) to activation of NF-□B. We are combining these advanced imaging techniques with molecular biology and biochemical approaches to determine the mechanistic relationship between microscopically defined protein redistribution events and biochemically defined TCR- and BCR-directed activation events. Our goal is to understand the mechanistic significance of protein redistribution events for signal transmission from the antigen receptor to NF-kB. Additionally, we are characterizing the spatial and temporal redistribution of T cell co-stimulatory molecules. We are particularly interested in determining the mechanism whereby the costimulatory molecule CTLA-4 inhibits T cell activation, including activation of NF-kB. We are also beginning to explore the link between translocations involving Bcl10 and MALT1 and a type of cancer called MALT lymphoma. Both Bcl10 and MALT1 are intermediates in TCR- and BCR-activation of NF-kB, and these proteins directly interact with each other. In addition to defining the significance of this interaction for signal transduction, we are also testing the hypothesis that translocation associated changes in the expression level of the Bcl10 protein and in the structure of the MALT1 protein play a major role in the etiology of MALT lymphomas. Finally, we have obtained several strains of knockout mice lacking specific genes that are essential to antigen receptor activation of NF-□B. We are now initiating collaborations with other investigators at USUHS, including Dr. Stephen Davies, to better understand the role of antigen-directed activation of NF-kB in the host immune response to pathogen infection.

Schmaljohn, Connie, Ph.D. Adjunct Faculty, Chief Scientist (ST)
U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)
Research Description: Research involves studies that lead to an understanding of mechanisms of replication, antigenic structure, or virulence properties of highly pathogenic human viruses, and ultimately to means for preventing or treating diseases. Current efforts include (1) developing multiagent and multiepitope DNA-based vaccines for highly hazardous viruses; (2) identifying key polymerase gene regions involved in replication; (3) elucidating mechanism(s) of interferon antagonism by hemorrhagic fever viruses; and, (4) developing novel antivirals for hemorrhagic fever viruses. BSL2, BSL3 and BSL4 containment laboratories are used as required to conduct these studies.

Snapper, Clifford, M.D., Professor
Department of Pathology
Research Description: Infections with extracellular (pyogenic) bacteria represent a major source of morbidity and mortality in the U.S. Humoral immunity to extracellular bacteria is conferred by both polysaccharide (PS)-specific and protein-specific IgM, IgG, and/or IgA which mediate opsonophagocytosis and/or complement mediated lysis, or prevent attachment to epithelial surfaces. The regulation of PS versus protein specific Ig responses is distinct. The need for immunotherapeutic approaches to control and eradicate these infections has become more compelling since antibiotic resistance in these pathogens has increased. The focus of our lab is the determination of the parameters that regulate the *in vivo* murine Ig isotype response to extracellular bacteria, using intact *Streptococcus pneumoniae* as a model microorganism. Ig isotype production specific for both the capsule PS and cell wall proteins in response to intact bacterial challenge is studied. Knockout and transgenic mouse models and blocking and stimulating monoclonal antibodies are employed. The role of T cells and dendritic cells, costimulatory molecules, the CD40/CD40-ligand interaction and cytokines are studied. ELISA ELISPOT, flow cytometry, immunohistochemistry, adoptive cell transfers, and “real-time” cytokine-specific RT-PCR are among the techniques used. Many aspects of basic immunologic processes are addressed in understanding this complex response. The information generated by these studies should prove useful towards the rational design of immunotherapies against these pathogens.

Stewart, Ann, Ph.D., Professor
Department of Immunology, Walter Reed Army Institute of Research
Research Description: The research involves analysis of cellular and humoral immune responses to malaria vaccine candidates and the investigation of mechanisms of protection in non-human primate model systems. Anti-malaria vaccines comprise components of pre-erythrocytic and erythrocytic-stage antigens that are expressed in a variety of systems, including recombinant proteins delivered in several adjuvants, and parasite genomic information delivered either "naked" or vectored in a modified virus. We analyze immune responses to various vaccine formulations and combinations in murine, simian, and human systems in order to optimize formulations for further development. We are specifically engaged in analysis of combinations of delivery strategies in a heterologous way, i.e.'prime-boost' vaccine strategies, in which, for instance, a malaria gene might be delivered first in a viral vector and second as a recombinant purified protein. The work in the laboratory involves the design, execution, and analysis of large pre-clinical trials to optimize vaccine formulations and delivery systems and for the careful documentation of safety and immunogenicity data to support clinical development of candidate vaccines. In addition, there are opportunities to study the fine details of the development of the immune response and the characteristics of the responses generated by different adjuvants or formulations.

Stoute, Jose, M.D., Associate Professor
Department of Medicine
Research Description: Pathogenesis:
Severe Malaria in Humans: This work is centered on expanding the understanding of the pathogenesis of severe malarial anemia and cerebral malaria in children living in western Kenya. Using case-control studies based in local hospitals we have identified differences in the level of expression of erythrocyte complement regulatory proteins between children with severe malaria and their controls that I feel shed light into the pathogenesis severe malaria. Current efforts are centered around measurement of functional differences between erythrocytes of children with severe malaria and their controls such as differences in the immune complex binding capacity as well as in the susceptibility of erythrocytes to complement-mediated damage. This work is currently supported by RO1HL7502-01 and Fogarty Training Grant 1D43TW06239-01, 2006.

Severe Malaria in Rodents: These studies are just getting underway at USU. The objective is to determine the contribution of complement and complement regulatory proteins to the pathogenesis of malaria in mice. For this purpose, we will use mouse knockouts of complement regulatory proteins and C3 to determine the impact of deficiency of these proteins on the course of malaria in mice. We have available several strains of rodent malaria that we will study in this study. Knockout mice will be provided by collaborators.

Erythrocyte Invasion: The invasion of the erythrocyte by malaria parasites is a highly complex process that is poorly understood and therefore, it is potentially a fruitful area of research. Sialic acid on glycophorins is felt to be the major ligand for plasmodium merozoites. However, an alternative pathway exists that is independent of sialic acid. The erythrocyte receptors as well as the parasite ligands involved in the sialic acid-independent invasion pathway are unknown. Our studies are geared towards the identification of sialic acid-independent receptors on the erythrocyte surface that serve as invasion receptors for malaria merozoites. For this purpose two strategies will be used. One strategy will involve the use of purified erythrocyte surface receptors either commercially available or purified in the laboratory to inhibit the sialic acid-dependent invasion. An alternative strategy will utilize tryptic digests of the erythrocyte surface to identify molecules that inhibit sialic acid-independent invasion. In order to identify parasite molecules that are potentially involved in sialic acid-dependent invasion we will carry out differential expression profiling of parasite strains grown in neuraminidase-treated (sialic acid-depleted) erythrocytes and normal erythrocytes.