

**Research Interests of
Training Faculty**

**Emerging Infectious Diseases
Uniformed Services University**

2008

Broder, Christopher, Ph.D., Professor, Department of Microbiology and Immunology; Director, Emerging Infectious Diseases graduate program.

Research Description: We are pursuing structural and functional analyses on the interactions between enveloped viruses and their cellular receptors using immunological, biochemical, and genetic approaches. Viruses under investigation in the laboratory include HIV-1, new or emerging paramyxoviruses; Hendra, Nipah, J-virus, and Beilong virus; Australian bat lyssavirus a rhabdovirus; and filoviruses (Ebola and Marburg). 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, and the discovery of new viral receptors. We are also interested in the structure of these viral envelope glycoproteins as well as the receptors they employ for host cell infection. We also place a particular emphasis on the immunological characteristics of the native viral glycoproteins including the potential to induce neutralizing antibody responses in vivo and the identification and characterization of neutralizing epitopes within the glycoproteins. To accomplish this, a variety of tools are used in the laboratory including animal cell expression and purification of native and soluble versions of the viral membrane glycoproteins, cell-cell reporter gene fusion assays, a variety of viral Env-pseudotyping platforms to study and quantify viral entry, murine monoclonal antibody development, and human monoclonal antibody discovery using antibody phage-display techniques. Both active subunit vaccine immunogens, based on viral Envs, as well as passive therapeutics such as neutralizing human monoclonal antibodies and fusion inhibitors are also derived from our studies. For example, several new and modified HIV-1 primary isolated-derived, soluble gp140 Env trimers are being studied as potential vaccines for their ability to elicit broadly cross-reactive neutralizing antibody responses in animals; work being carried out in collaboration with Dr. Gerald Quinnan (USUHS). Hendra and Nipah virus are particularly interesting because of their broad species tropism and highly pathogenic nature, and each virus has continued to re-emerged causing human and animal deaths. Potential antiviral therapeutics have been developed against Hendra and Nipah virus and are being evaluated in a variety of animal models. These studies that involve Nipah virus and Henda virus are conducted collaboratively with several scientists located at CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong, Australia, one of only a handful of BSL-4 facilities equipped to work with these agents using larger animal models.

**Burgess, Timothy, MD, MPH; CDR, MC, USN, Adjunct Faculty
Chief, Viral and Rickettsial Diseases Department. Naval Medical Research Center**

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.

**Burns, Drusilla, Ph.D., Adjunct Faculty
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 interfere 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 Multidrug Transporter: Mode of Action and Functional Regulation: The effectiveness of anti-microbial and anti-cancer chemotherapy largely depends on the ability of the therapeutic 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, the therapeutic 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 wide range of structurally unrelated hydrophobic drugs including anti-cancer and anti-HIV agents. In order to retain the therapeutic 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 and endogenous molecules and 3) identification of novel therapeutic targets in the protein. We use a vaccinia virus mediated infection/transfection protocol for generation of recombinant Pgp molecules and 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* was the first bacterium classified as a class I carcinogen by the WHO and is now established as a necessary, but not sufficient cause of gastric cancer. My lab focuses on the respective roles of *H. pylori* and of dietary factors in gastric epithelium and lymphocyte oncogenesis. Although *H. pylori* is primarily colonizing the lumen of the stomach, we and others have demonstrated that *H. pylori* can be detected inside metaplastic, dysplastic, and neoplastic epithelial cells, and that the fraction of bacteria expressing the virulence factor *cagA in situ* is higher in precancerous and cancerous lesions than in controls. These novel findings are compatible with the hypothesis that early and late 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. Regarding the role of the diet, we have recently observed that gastric cancer can be induced by a combination of *H. pylori* plus a dietary carcinogen but not by either factor alone. We are currently studying dietary isoflavones, compounds that have anti-inflammatory, antimicrobial, antiangiogenic, and anticarcinogenic effects. Current studies are analyzing the separate and combined effects of *H. pylori* and dietary factors on gastric histopathology, mucosal and peripheral T cell populations and cytokine expression, and concurrent gastric host and bacterial gene expression.

Giam, Chou-Zen, Ph.D., Professor

Department of Microbiology and Immunology

Research Description The research in my lab focuses on the molecular biology and pathogenesis of three human cancer viruses: human T-lymphotropic virus type 1 (HTLV-1), Kaposi sarcoma-associated herpesvirus/human herpesvirus type 8 (KSHV/HHV-8), and hepatitis C virus (HCV). We are particularly interested in how regulatory proteins of these viruses usurp cellular mRNA transcriptional machineries, cell cycle control mechanisms, and signal transduction pathways to facilitate viral replication. We have a long standing interest in the mechanisms of action of the HTLV-1 transactivator/oncoprotein, Tax, and its role in the etiology of adult T-cell leukemia (ATL). A major research effort concentrates on the mechanisms by which Tax activates I- κ B kinases and T-cell transformation. We have found recently that Tax can activate an E3 ubiquitin ligase, the anaphase promoting complex/cyclosome (APC/C), ahead of schedule. This activity of Tax causes premature degradation of many critical mitotic and cell cycle regulators during S phase, leading to DNA aneuploidy and cellular senescence. Genetic and biochemical approaches are being used to study the mechanism by which Tax activates APC/C and how this activity impacts on chromosome instability and cellular senescence. Using reporter cell lines, we have found that HTLV-1 infection indeed leads to a senescence-like cell cycle arrest, contrary to the prevailing paradigm which maintains that HTLV-1 causes proliferation of infected T cells leading to leukemia. Another major effort is to test the hypothesis that an evasion of cellular senescence after HTLV-1 infection (through specific cellular mutations) constitutes a critical step in ATL development. The reporter cell lines that can detect HTLV-1 infection are also being utilized to assess the roles of other HTLV-1 accessory genes in viral replication and transmission. Proteomic and virological approaches are also being used to study the structures and functions of two regulatory proteins, K-Rta and K-bZIP of KSHV/HHV-8, with a special emphasis on their roles in viral replication and latency reactivation. Finally, a study to investigate the mechanism of hepatitis C virus (HCV) replication has been initiated in 2003. Current efforts focus on identifying cellular factors important for HCV replication.

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 area 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* and there are no licensed vaccines against this organism. Research is focused on characterization of surface antigens of *C. jejuni* and understanding their role in virulence with the ultimate goal being vaccine development. *C. jejuni*, unlike other enteric pathogens, expresses a polysaccharide capsule. The structure of this capsule, which undergoes high frequency phase variation, is highly variable among strains and is required for invasion of epithelial cells in vitro and virulence in a ferret model of diarrhea. Current emphasis is on defining the specific role of capsular polysaccharides in the disease process and determining if structural differences among capsules affect virulence. Additionally, flagella are multi-factoral virulence factors of *C. jejuni*. The flagellins are heavily glycosylated with unusual 9-carbon sugars (pseudaminic acid and derivatives) that resemble sialic acid. Changes in the glycans that modify flagellin affect invasion of epithelial cells, and we are interested in defining the roles that these glycans play in host-cell interactions. We are also characterizing several novel non-flagellar proteins that are co-regulated with the flagella regulon that contribute to virulence of *C. jejuni*.

Huggins, John W., Ph.D., Adjunct Faculty

Chief, Viral Therapeutics Branch, Virology Division, (USAMRIID)

Research Description: Antiviral Drug Development focusing on 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 utilized under the FDA Animal Efficacy Rule. Toward that goal we will continue studies to better characterize the disease and to evaluate new oral antiviral drugs that are active against orthopoxviruses. Our laboratory also supports an ongoing clinical trial 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., 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.

Kaleeba, Johnan, Ph.D., Assistant 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, NMRC-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. NMRC-Lima 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

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 (including microRNA expression) by which they exert this effect. For more information, please see *J Virol* 2007 81:1872-8, *J Virol*. 2007 81:6605-13, and *Proc Natl Acad Sci U S A* 2008 105:10931-6. 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. These types of techniques have potential utility in virus discovery, studies of disease pathogenesis, bioterrorism and biological warfare preparedness, and regulatory applications. For more information, please see *J Virol Methods* 2008 152:18-24.

Mattapallil, Joseph, B.V.Sc., M.S., Ph.D., Assistant Professor

Department of Microbiology and Immunology

Research Description: HIV infection is a leading cause of death in the world. According to WHO, almost 45 million people have been infected with HIV. 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 (Mattapallil et al *Nature* 2005, *J. Exp. Med.* 2006). 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. Not much is known about the early events that drive host-pathogen interactions, and the molecular interactions that occur leading to the massive replication of the virus. Understanding these early events is a major objective of our laboratory as it will give us new insights early pathogenic events, but also will help us design better therapeutic and vaccine strategies. We rely on both cellular (multi-color flow cytometry) and molecular (quantitative and relative Taqman PCR, micro-array's) tools to address the various questions related to HIV and Herpes virus (EBV) infections. Current ongoing work focuses on (1) understanding the role of innate and adaptive cytokine responses in early host-pathogen interaction and viral amplification, (2) delineating the mechanisms of CD4 T cell depletion in HIV infection, (3) understanding the mechanisms that drive the reactivation of EBV infection in the oral cavity leading to oral cancer, (4) identifying molecular biomarkers of HIV and EBV disease progression using microarray and proteomic tools, (5) development of vaccines against HIV infection using DNA/MVA vaccines. These studies use both in vitro approaches, and in vivo infections using the non-human primate models such a rhesus macaques. These studies will significantly advance our knowledge about the cellular and molecular mechanisms of viral pathogenesis, and help in the development of better therapeutic and vaccine approaches to control HIV and other related viral infections.

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 trypanosomiasis, 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 typanosomes will be lethal.

Merrell, D. Scott, 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.

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 hypo-responsive 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 do this, our lab utilizes the *Litomosoides sigmodontis* model of filaria infection, the only mouse model of filariasis in which larvae fully complete their development from infective L3 stage larvae into mature, sexually reproducing adult filarial worms. In addition to mouse immunology, we also have an ongoing collaboration with investigators at NIH in which we are trying to determine the underlying mechanisms of immune deficiency in patients with the hyper-IgE syndrome. Finally, we have recently demonstrated that *L. sigmodontis* worms prevent the development of type 1 diabetes in NOD mice and are actively engaged in determining the mechanisms by which this protection occurs.

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, evaluation 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 selected HIV-1 envelope glycoproteins as immunogens, using various methods for in vivo expression and delivery of soluble glycoproteins in adjuvants. Accomplishments include inductions of cross-reactive neutralizing antibodies in monkeys that protected them from intravenous challenge with heterologous strains of challenge virus, and the induction of broadly cross-reactive neutralization of resistant primary viruses. Studies are in progress to further define the glycoprotein characteristics important for these responses and the potential for more substantial induction of B cell responses leading to more potent and broadly protective immunity.

Richards, Allen, Ph.D., Associate Professor
Department of Preventive Medicine and Biometrics

Research Description: Major research interests are in the study of arthropod-borne diseases, especially rickettsial diseases, utilizing epidemiology, immunology, and vaccine and rapid diagnostic assay development to decrease the risk of arthropod-borne diseases detrimental affect on military and civilian populations. While developing a broadly protective DNA vaccine for scrub typhus we are elucidating the mechanisms of the host immune response that play a role in controlling the infection of *Orientia tsutsugamushi* initially at the bite site of the vector mite as well as during the spread of the infection throughout the body. In association with these studies we are working on identifying a surrogate marker for immunoprotection. Other interests include the development of real-time PCR assays to detect agents of arthropod-borne diseases for diagnostic assay development, monitoring vaccine efficacy in NHP trials, and identification and enumeration of agents within arthropod vectors in risk assessment studies conducted both in the States and overseas.

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- κ B. 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- κ 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- κ 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- κ B. 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- κ B. 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- κ B, 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- κ 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- κ B in the host immune response to pathogen infection.

Schmaljohn, Connie, Ph.D. Adjunct Faculty, Chief Scientist (ST) (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. In addition, we are involved in developing new technologies for malaria diagnosis in the field and in the laboratory, and in evaluating some aspects of the interaction of HIV and malaria in the field.

**Via, Charles S., M.D., Professor
Department of Pathology**

Research Description: My laboratory research effort uses the parent-into-F1 model of graft vs. host disease (GVHD) as an *in vivo* model to study the development of cytotoxic T lymphocytes (CTL) and the immunopathogenesis of systemic lupus erythematosus, a humoral autoimmune disease that affects primarily young females. GVHD is induced by the transfer of homozygous parental strain T cells into normal F1 mice. Depending on the murine strains used, disease takes one of two outcomes: a) acute GVHD mediated by donor CTL that attack host tissues and b) chronic GVHD, a disease that strongly resembles human lupus. Like naïve T cells, CTL require two signals for activation – an antigen specific signal mediated through the T cell receptor and a second costimulatory signal mediated through CD28 initially. These two signals result in proliferation however maturation to effector CTL also requires a third signal that can be delivered by cytokines. Importantly,

defects in CTL development convert acute GVHD to lupus-like chronic GVHD. Conversely, agents that promote CTL convert lupus-like chronic GVHD to acute GVHD. Lastly, lupus-like disease occurring in chronic GVHD mice is more severe in females just as human disease. Our current efforts are focused on: 1) defining the mechanisms responsible for sex-based differences in lupus-like disease; 2) determining the consequences of defects in Fas/FasL and perforin mediated killing on the subsequent development of lupus; 3) defining the role of T cell downregulatory molecules (e.g., Fas, CD80, PD-1) and their suitability as targets for enhancing or reducing CTL function in vivo; 4) defining cytokines and agents that can either deliver signal 3 or induce signal 3 mediating molecules for CTL maturation. Agents identified as having CTL promoting abilities will be tested in spontaneous models of murine lupus for their therapeutic value; and 5) identifying additional cell surface molecules and cytokines with critical roles in enhancing or downregulating CD8 CTL responses. Our laboratory uses multi-color flow cytometry extensively. We have also adapted an in vivo cytotoxic killing assay using non-radioactive parameters. Other methodology used extensively includes real time PCR, ELISA, immunohistology and routine histology. We are developing expertise in confocal microscopy.