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Research Interests of Training Faculty Emerging Infectious Diseases

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Kimberly Bishop-Lilly, Ph.D., Adjunct Faculty
Deputy Head of Genomics Department, Naval Medical Research Center (NMRC)-Frederick, MD
Biological Defense Research Directorate (BDRD); Adjunct Assistant Professor.
Research Description: The mission of the BDRD Genomics Department is to apply bioinformatics and/or cutting-edge, high-throughput technologies -such as genotypic and phenotypic expression profiling, biomarker characterization, and bacteriophage expression systems - to the study of infectious diseases, biodefense, and enhanced global disease surveillance for early pathogen detection and response. Current research and development projects include 1) metagenomic sequencing of environmental and vector samples to support biosurveillance, novel virus discovery, characterization, assay design, and countermeasures; 2) approaches for pathogen enrichment to enhance sequencing-based detection and characterization; and 3) genetic characterization of novel therapeutic bacteriophages. Other interests include bats as viral reservoirs.

Christopher C. Broder, Ph.D., Professor and Chair
Department of Microbiology and Immunology
Research Description: We are pursuing structural and functional analyses on the interactions between enveloped viruses and host cells using immunological, biochemical, and genetic approaches. Viruses under investigation include the henipaviruses Hendra, Nipah Cedar and Mojiang viruses; Australian bat lyssavirus 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 develop and characterize soluble, native-like, oligomeric viral glycoproteins and examine their immunological characteristics including the potential to induce neutralizing antibody responses in vivo. These recombinant viral glycoproteins are also being used in several biosurveillance studies for the detection of antiviral antibodies in wildlife, livestock and humans. A variety of tools are used in the laboratory including animal cell expression and purification of viral membrane glycoproteins, cell-cell reporter gene fusion assays, monoclonal antibody development and viral reverse genetic systems. We are also interested in both vaccine and monoclonal antibody therapeutics development. 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 and vaccines have been developed against Hendra and Nipah virus in collaboration with scientists located at CSIRO, Livestock Industries, Australian Animal Health Laboratory, Geelong, Australia, and scientists at the Galveston National Laboratory, University of Texas Medical Center, Galveston, Texas. Our Hendra G glycoprotein subunit vaccine (HeV-sG) is marketed in Australia, manufactured by Zoetis, Inc., (Equivac® HeV) to prevent Hendra virus infection of horses and break the transmission of Hendra to people. We are currently developing HeV-sG as human vaccine for Hendra and Nipah viruses in collaboration with Profectus Biosciences and now supported by CEPI. A Phase I clinical trial of the anti-Nipah/Hendra human monoclonal antibody (m102.4) was completed in 2016. m102.4 has proven successful in nonhuman primates to provide compete post-exposure protection against both Nipah and Hendra virus infection and disease, and has been administered to 14 individuals on an emergency use basis in Australia and the US.

Stephen Davies, Ph.D., Associate 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's 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 the host immune system can mediate at least some protection 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 and macrophages to complete their development normally, suggesting that blocking interactions between schistosomes and host 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 host 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.

Saibal Dey, Ph.D., Associate 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 depends on the ability of the therapeutic to reach their sites of action. Following administration, the fate of a drug 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, development of chemotherapeutic drugs has to continuously battle against poor bioavailability and 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.

J. Stephen Dumler, M.D., Professor and Chair
Joint Departments of Pathology, Uniformed Services University and Walter Reed National Military Medical Center (WRNMMC), Bethesda, MD
Research Description: The laboratory is predominantly set to investigate vector-borne disease pathogenesis, with a strong emphasis on tick-borne infectious agents and intracellular bacteria in the order Rickettsiales. We employ multidisciplinary approaches toward scientific investigation ranging from simple diagnostic methods for clinical purposes, to molecular, genomic and epigenetic approaches for fundamental scientific discovery. Most basic work focuses on *Anaplasma phagocytophilum* AnkA, a type IV secretion system substrate that epigenetic regulates host chromatin and nuclear structure to influence eukaryotic gene transcriptional programs by binding gene promoters in cis and by binding chromatin into the nuclear

lamina in trans. Additional investigations in the lab focus on i) *Rickettsia, Anaplasma, Ehrlichia,* and *Borrelia* infections and immunopathology mediated by NKT and cytotoxic T cells; ii) *A. phagocytophilum* immunopathology related to NKT, NK, and CD8 T cells immune responses; iii) Stat1 signaling and its role in immunopathology with *A. phagocytophilum* infection. We also study tick-borne co-infections and their synergistic pathogenetic mechanisms with Lyme disease and human granulocytic anaplasmosis. Because an underlying tenet of infection pathogenesis with many vector-borne pathogens is increased vascular permeability or access to the vasculature for systemic dissemination, we also study, pathogen endothelial cell barrier intravasation, invasion, dissemination, extravasation, and vascular permeability by *Borrelia, Rickettsia, Ehrlichia* and other pathogens *in vitro* and *in vivo.* These studies focus in part on the roles of intracellular calcium concentrations, divalent cation chelators, phospholipase C inhibitors and antagonists of signaling through G protein-coupled receptors that prevent increases in vascular barrier permeability. Finally, the laboratory supports clinical investigations of febrile disease etiology in under-resourced regions, including Malaysia, Sri Lanka, Nicaragua, Tanzania, among other locations. These studies focus on high-throughput and multiplexed methods for molecular diagnosis and avoidance of misdiagnosis to prevent inappropriate treatment and the consequent negative outcomes. The aims are to i) answer how much fever in such regions is the result of undiagnosed rickettsial disease, and ii) implement clinical studies and trials of new diagnostic tests, therapies and vaccines to prevent these vector-borne infections.

Kristi L. Frank, Ph.D., Assistant Professor
Department of Microbiology and Immunology

Research Description: Pathogenesis of biofilm-associated infections — Bacterial biofilms are highly-ordered populations of cells attached to a surface, or each other, and are encased in a self-produced extracellular matrix. Biofilm growth provides protection from adverse environmental conditions, enables evasion of the host immune defenses, and confers resistance to remarkably high concentrations of antimicrobial agents. Our group studies relationships between bacterial pathogens and their hosts in biofilm-associated infections. In particular, our efforts focus on *Enterococcus faecalis*, a Gram-positive bacterium that is both a human commensal and an opportunistic pathogen that has emerged as a leading cause of healthcare-associated infections. In immunosuppressed patients, *E. faecalis* can cause endocarditis, bloodstream infections, surgical site infections, and catheter-associated urinary tract infections. The Frank Lab pairs animal models of biofilm formation with genetic, molecular, and biochemical approaches to (1) define sensing pathways and regulatory circuits that are involved in triggering transcriptional and stress responses in the host environment, (2) identify biofilm-associated virulence factors in *E. faecalis* and determine how they affect interactions between the bacterium and its host, (3) devise new methods to remove biofilms and prevent their formation, and (4) pursue the mechanisms by which *E. faecalis* colonizes endovascular tissues as part of a broader interest in understanding how biofilm-forming bacteria may affect human cardiovascular health.

Chou-Zen (Joe) Giam, Ph.D., Professor
Department of Microbiology and Immunology

Research Description: The work in my lab revolves around human viruses, with a special emphasis on human T-cell leukemia virus type 1 (HTLV-1), and with occasional forays into other viruses such as HIV and KSHV. We seek a mechanistic understanding of how viral regulatory proteins hijack cellular mediators of mRNA transcription, cell cycle control, and signal transduction to effect viral replication and pathogenesis, especially, oncogenesis. We (and others) have elucidated the mechanisms by which the HTLV-1 transcriptional activator/oncoprotein Tax activates viral transcription and I-κB kinases/NF-κB signaling, and contributed to current understanding of how HTLV-1 infects, replicates and causes diseases. We are interested in the basic principles of HTLV-1 replication, persistence, and pathogenesis, and strive to translate basic science findings into preventive measures and therapeutic approaches for HTLV-1-associated diseases, especially adult T cell leukemia (ATL). We also use the HTLV-1-ATL system as a model to understand the pathogenesis of other NF-κB-addicted human lymphoma/leukemia, and microbial infection-associated human cancer. Our current efforts focus on (1) elucidating the mechanisms that

underlie the genomic instability of ATL cells; (2) understanding how the transcriptional activity of NF-κB drives the induction of cellular senescence; (3) using whole genome sequence information to identify the genetic alterations in ATL cells that mitigate the senescence response driven by NF-κB and promote ATL developments; and (4) uncovering the mechanisms that regulate HTLV-1 latency and reactivation.

Ann E. Jerse, Ph.D., Professor and Vice Chair
Department of Microbiology and Immunology

Research Description: Pathogenesis of Neisseria gonorrhoeae and development of products against gonorrhea: Gonorrhea is a very common bacterial infection of the genital tract, rectum and phayrnx. Ascending infections can damage the reproductive tract, particularly in women, and babies born to infected mothers can acquire gonococcal conjunctivitis and may have reduced birth weight due to premature rupture of membranes. Gonorrhea also increases the spread of human immunodeficiency virus (HIV). The rapid evolution of antibiotic resistance in N. gonorrhoeae threatens current control measures and there is no gonorrhea vaccine. Research in the Jerse lab centers around the use of a female mouse model of gonococcal infection that we developed to facilitate studies in a whole model system. Current research areas include i.) gonococcal adaptation to innate host defenses with an emphasis on evasion of antimicrobial peptides; ii.) impact of antibiotic resistance mutations on gonococcal fitness in vitro and in vivo and identification of compensatory mutations that may facilitate persistence of highly resistant organisms; iii.) refinement of the gonorrhea mouse model by alleviating host restrictions iv.) animal modeling of N. gonorrhoeae and Chlamydia trachomatis coinfection; v.) development of gonorrhea vaccines that target surface molecules involved in evasion of host defenses. We also conduct pre-clinical testing of candidate antibiotics, vaginal microbicides and vaccines against gonorrhea in collaboration with academic colleagues and pharmaceutical companies.

M. Gordon Joyce, Ph.D., Adjunct Faculty
Chief, Structural Biology, US Military HIV Research Program, Walter Reed Army Institute of Research (WRAIR), Silver Spring, M.D.

Research Description: Our lab focuses on viral antigen-neutralizing antibodies, and the structure-based design of HIV-1, Influenza, flaviviruses, and MERS glycoprotein vaccine candidates. We study high resolution structural details of vaccine-host-virus interactions e.g. antibody-antigen interactions, and how antibodies evolve to increase potency and efficacy. Our group works closely with collaborators at WRAIR, NIH, and other research institutes to characterize human and animal immune responses following infection or vaccination. Most recently, we have been involved in the study of the induction and prevalence of broadly protective antibodies in humans against Zika virus, and influenza and utilizing this information, to carry out structure-based design of vaccine candidates. This process relies on four inter-dependent parameters: protein design, antigenic analysis, structural analysis, and animal immunogenicity studies. In addition to emerging infectious diseases, we are focused on understanding the immune response to HIV-1, and developing HIV-1 vaccine candidates. HIV-1 minimizes antibody responses to its surface glycoprotein Env, through structural malleability, sequence diversity, and glycan shielding. Despite these evasion mechanisms, there are specific antigenic sites of vulnerability on the viral Env molecule including the CD4-binding site located on the outer domain. Significant progress has been made in developing immunogens based on the HIV-1 Env molecule including stabilized trimeric immunogens, and engineered outer domain molecules. This has been accomplished via structure-based design, and validation, leading to the elicitation of neutralizing antibody responses in small animal and non-human primate studies. Multiple successful structure-based designs including HIV-1 outer domain molecules, single-chain HIV-1 Env molecules, and fully-cleaved HIV-1 Env molecules from >80 diverse strains have been designed and validated as antigenic mimics of the native HIV-1 viral Env. Antibodies with protective activity are thought to be a critical component of an effective HIV vaccine. Characterization of B cell and virus co-evolution in patients, is also informing our understanding of antibody development. In order to counteract HIV-1 viral diversity, broadly neutralizing antibodies precisely target specific sites of vulnerability. Structure-based vaccine design allows the development of novel immunogens that can target the induction and development of specific antibodies

targeted to sensitive supersites. Our lab researches the interplay between HIV Env structure and function including Env sequence diversity, glycan shielding and structural malleability and how this information can be used to develop novel Env-based immunogens capable of eliciting broadly protective antibodies.

Philip Krause, M.D., Adjunct Faculty
Deputy Director, Office of Vaccines Research and Review, CBER/FDA
Research Description: Viral latency is a central issue in considerations of vaccine safety and in consideration of vaccine efficacy against viruses that may establish latency. In addition, the ability to establish latency provides viruses with an evolutionary advantage, allowing spread through the lifetime of an infected individual. Our laboratory is investigating the molecular pathogenesis of herpes simplex virus latency, with an emphasis on HSV-2. We have identified viral sequences that control reactivation phenotypes and have learned that the virus employs a variety of molecular strategies that combine to control the outcome of viral infection within an individual cell. These include expression of microRNAs, use of alternative splicing, and direct inhibition of viral transcription through promoter sequences. We also have been identifying key features of immune responses that control virus infections with the goal of determining how these responses may be further improved. In performing these experiments, we rely heavily on construction and evaluation of mutant viruses (including animal models), the full range of molecular virology techniques, and high throughput sequencing.

George W. Liechti, Ph.D., Assistant Professor
Department of Microbiology and Immunology
Research Description: The major research interest of our laboratory is the molecular genetics of bacterial pathogenesis. We focus on organisms of the genus Chlamydia, which is comprised of obligate, intracellular pathogens that can cause a variety of diseases including pneumonia, blinding eye infections, and sexually transmitted diseases. Pathogenic *Chlamydia* is currently the 'silent epidemic' of our time, with approximately 3% of young women between the ages of 15-19 infected in the US, and between 90-300 million infected globally. Despite being first characterized over a century ago, relatively little is known about this human-adapted microbe's most rudimentary cellular functions. We are in the process of developing an assortment of molecular and genetic approaches to help increase our understanding of chlamydial biology in the hopes of identifying pathways that are essential for *Chlamydia* growth, which can provide new targets for future drug development. Our laboratory recently identified the major bacterial cell wall component peptidoglycan in pathogenic *Chlamydia*, resolving a longstanding 'anomaly' concerning the microbe's basic physiology. We are currently investigating the steps involved in the synthesis and degradation (turnover) of peptidoglycan in *Chlamydia*, its role in the developmental cycle of the organism, how it functions in conjunction with the microbe's cellular division machinery, and to what extent it interacts with the mammalian host's immune system. We are also actively investigating the mechanisms by which pathogenic *Chlamydia* persist in their mammalian hosts and characterizing several transporters essential to the microbe's ability to uptake nutrients and sense its intracellular environment.

Allison Malloy, M.D., Assistant Professor
Department of Pediatrics
Pediatric Infectious Disease Faculty
Research Description: Respiratory viruses cause tremendous morbidity and mortality throughout life. Viral load alone often does not predict severity of disease, but rather is dependent on the host response. Respiratory syncytial virus (RSV), in particular, exemplifies this paradigm whereby severity of disease is associated with age upon infection. During the first year of life RSV is the single most important respiratory pathogen and globally causes the greatest mortality. Despite 80-90% of the population becoming infected during the first 2 years of life, repeated infection throughout life is common demonstrating that RSV evades sterilizing immunity. Moreover, after the age of 65 years RSV again results in increased severity of disease and mortality. By evaluating the age-dependent cellular and molecular weaknesses in host response to

respiratory viral infection we can identify critical immune defense mechanisms to inform intelligent design of therapeutics and vaccines. Innate immune cells that present antigens derived from virus determine the emergence of the adaptive immune response. Using experimental murine models, immune cell isolation and culture, flow cytometry, RNA-seq and other state-of-the-art techniques, we have shown that RSV-specific antibody, cytotoxic T cells, and conventional and regulatory T cell responses are shaped by antigen presentation and signaling events associated with immune synapses. Our lab seeks to define the antigen-specific innate interactions, particularly those involving dendritic cells, that support T cell activation and effective adaptive responses, as well as those responses that induce tolerance or autoimmunity in order to better target, or avoid, this engagement through vaccine delivery. Additional future work aims to use animal model findings to identify triggers of protective immunity in human pediatric and adult respiratory viral infection.

Joseph Mattapallil, B.V.Sc., M.S., Ph.D., Associate Professor
Department of Microbiology and Immunology
Research Description: The primary research in my laboratory focuses on host virus interactions with the intent of understand the correlates of pathogenesis and using these correlate to develop better therapeutic and vaccine approaches. More specifically our efforts have centered around emerging viruses such as Zika and Dengue, and HIV infection. We have recently shown that the interplay between Zika and Dengue immune responses have implications for the development of vaccines as th immune responses induced by Zika infection appear to enhance infection with Dengue viruses (George et al Nature Scientific Reports 2017; Valiant WG et al Emerging Microbes and Infection 2018; Valiant WG Journal of Visual Experiments 2018). We have primarily relied on using the non-human primate model for these studies along with the various cell lines that are susceptible to both Zika and Dengue viruses. We were able to translate our findings using serum from human subjects who had traveled to Latin America and were infected with Zika virus to show that prior infection with Zika has the potential to significantly enhance Dengue infection *in vitro (Valiant WG Open Forum Infectious Diseases 2018)*. Currently studies are underway to characterize the nature of immune responses induced by Zika and identify specific correlates that can be used to develop a second generation vaccine that can protect against Zika and Dengue without the adverse effects of enhancement.

A second focus of my laboratory is to develop strategies to achieve full functional cure from HIV infection. Functional cure refers to complete remission in the absence of anti-retroviral drugs. This has been a major challenge in the field and a priority area as there are currently about 35 million people who are living with HIV. Infection with HIV leads to integration into the host genome, a process that occurs very early in infection that 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 J et al Nature 2005, Mattapallil J et al J. Exp. Med. 2006; Kader et al Mucosal Immunology; Eberly et al Journal of Immunology). 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 and allow us to develop better therapeutic and vaccine strategies to get rid of HIV from infected cells in the body leading to a more durable functional cure for the infected patients.

Angela Melton-Celsa, Ph.D., Associate Professor
Department of Microbiology and Immunology
Research Description: We focus on bacterial pathogenesis of enteric infections and use enteroaggregative and Shiga toxin-producing *Escherichia coli* as model systems. Enteroaggregative *E. coli* (EAEC) are a leading cause of diarrhea in deployed military personnel (DPM) and represent a significant loss of man hours for the military. EAEC also cause acute and persistent diarrhea in children in developing

countries and in the immunocompromised. EAEC form biofilms and adhere in an aggregative pattern on host cells. However, EAEC are heterogeneous in terms of which additional virulence characteristics they express. We are assessing strains isolated from DPM for virulence traits and pathogenicity in a mouse model. Virulence factors identified from those EAEC will be assessed for the potential to serve as vaccine candidates. Shiga toxin (Stx)-producing *E. coli* (STEC) cause hemorrhagic colitis and the infection may result in a serious sequela, the hemolytic uremic syndrome which can be fatal. STEC can produce one or more Stxs and we are interested in the roles those toxins play in disease. We use a mouse model to evaluate the roles of Stx subtypes for disease in that animal model. In addition, we are interested in finding adherence factors for STEC that do not utilize the adhesin intimin for attachment.

D. Scott Merrell, Ph.D., Professor
Interim-Director, Emerging Infectious Diseases graduate program.
Department of Microbiology and Immunology
Research Description: Basic Biology of Bacterial Diseases, Human Microbiome, Bacterial Stress Response and Adaptation, Polymicrobial Interactions: The process of human-bacterial interaction is complex and 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 various types of disease. Currently, our studies are focused on several bacterial pathogens: *Helicobacter pylori*, *Staphylococcus aureus*, and *Acinetobacter baumannii*. All of these bacteria are important human pathogens that result in significant morbidity and mortality in infected individuals. Our ongoing studies are diverse and include the following: a) study of factors that allow *H. pylori* to colonize the human stomach and to induce gastric cancer/ulcers/intestinal metaplasia, b) *H. pylori* biofilm formation, c) microbiota associated with development of cancer, d) microbiota changes associated with *S. aureus* colonization and disease, e) *S. aureus*-mediated skin and soft tissue infections, f) mechanisms of resistance to antiseptics/resistance gene spread among *S. aureus* isolates, g) polymicrobial interactions as a means to inhibit *S. aureus*, and h) copper resistance in *A. baumannii*.

Nelson L. Michael, M.D., Ph.D.; COL (ret), MC, USA, Adjunct Faculty
Director, Center for Infectious Disease Research, Walter Reed Army Institute of Research
Research Description: I have been in the field of vaccine research since 1989, serving as director of MHRP from 2006-2018 and now direct all infectious disease research at WRAIR. This period included the completion of the RV144 HIV prime-boost vaccine study, an international collaboration that involved more than 16,000 Thai volunteers, and provided the world's first demonstration that a preventive HIV vaccine was possible. I have played a key role in the P5 partnership to build of the success of RV144 including publications in high-impact journals describing correlates of risk of infection and elucidation of viral sieve effects. We initiated a partnership with BIDMC/Harvard University to pursue and test novel Ad/MVA/gp140 vaccine candidates in harmonized NHP and clinical studies in partnership with Janssen and JnJ which advanced rapidly into efficacy studies that started in 2017. Our program has expanded into HIV therapeutics and cure research focused on our unique, large, and highly efficient very early acute HIV infection cohorts in Thailand and Africa and in NHP models in close collaboration with BIDMC inclusive of cART, Ad26/MVA, and LRAs. These preclinical studies have informed clinical studies using Ad26/MVA vaccination in very early AHI patients that started in March 2016 and will be read out by summer 2018. Recently, we have made significant strides in testing first in human Ebola vaccines in Africa and the U.S;. FIH studies of MERS-CoV vaccines in the U.S. and Jordan; Zika vaccine candidates. WRAIR has a range of world class laboratories in humoral and cellular and innate immunology, molecular virology, host genetics, structural biology, systems immunology, biochemistry, and animal models. My work on outside advisory committees —including President Obama's Presidential Commission for the Study of Bioethical Issues, the Vaccine Research Center Scientific Advisory Working Group (NIAID, NIH), Office of AIDS Research Advisory Committee (NIH), AIDS Research Advisory Committee and the PEPFAR Scientific Advisory Board—have drawn on and strengthened the depth of my experience and understanding of clinical research in a cross-cutting framework inclusive of bioethics to discovery and translational research and delivery of prevention

and treatment services. The totality of these experiences, along with the ability to adapt to changing external circumstances, help ensure that WRAIR will be an innovative scientific platform from discovery to infectious diseases countermeasure licensure to protect the Warfighter and the global community and an ideal training environment for graduate scientific education.

Edward Mitre, M.D., Professor
Department of Microbiology and Immunology

Research Description: Our laboratory studies the immunology, molecular biology, and pathogenesis of helminth infections and allergic diseases in order to develop new approaches to prevent and treat parasitic, allergic, and autoimmune diseases. The helminths we focus on are filariae, tissue-invasive vector-borne roundworms that cause debilitating diseases in millions of people worldwide. Pathogenic human filariae include *Wuchereria bancrofti* and *Brugia malayi*, which cause lymphatic filariasis (elephantiasis), *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. Interestingly, similar immunological changes are observed in allergic diseases, with type 2 immune responses driving allergic diseases and regulatory responses protecting against allergy after allergy desensitization. Our laboratory uses a variety of in vitro and in vivo models to study helminth infections. These include *Litomosoides sigmodontis*, a filarial nematode of rodents, and *Brugia malayi*, a filarial pathogen of humuans. To study allergy, we utilize cutaneous anaphylaxis, systemic anaphylaxis, and allergic asthma models. Current studies are focused on identifying novel drug and vaccine targets in filarial nematodes, evaluating natural products for the treatment of filariae, developing helminth-derived therapies for allergy and autoimmunity, and elucidating the mechanisms that drive immunoregulation in allergy immunotherapy.

Marzena Pazgier, Ph.D., Associate Professor
Department of Medicine

Research Description: We seek to understand, at the structural level, the mechanisms involved in adaptive responses to infectious disease with the long term goal to generate the knowledge required to develop new vaccines or antibody/protein therapeutics. Our research is highly interdisciplinary, involving structural biology, primarily by X-ray crystallography, contemporary biophysical and protein engineering techniques, and structure-function analysis. Projects in our laboratory are grouped under the following themes: a) Structural basis of Fc-mediated effector functions of antibodies against HIV-1. Fc-mediated effector function of antibodies is a mechanism whereby antigen-antibody complexes on free virial particle or virus-infected cell arm effector cells (phagocytes, NK cells, macrophages, etc.) enabling them to phagocyte/lyse the target virion/infected cell. In this project we primarily seek to understand the molecular basis of mechanisms governing broad and potent Fc-mediated effector function of antibodies against HIV-1 in human and non-human primates (NHP), Rhesus macaques. Our studies focus on how epitopes in the HIV-1 envelope glycoprotein (Env) elicit protective Fc-dependent responses and what are the molecular bases for the effective Fc γ receptor (FcγR) engagement in these processes. b) Development of new vaccines and epitope specific reagents. Our structural understanding of Fc-mediated anti-HIV effector function provides the basis for the development of new envelope constructs that we test in animals (primarily mice) as vaccines to elicit antibody response capable of Fc-mediated effector function. c) New strategies for a functional cure through antibody-dependent cell-mediated cytotoxicity. The apparent successes of cancer cure strategies that are based on therapeutic antibodies targeting immune checkpoints have caused a reevaluation of the potential for similar strategies for an HIV-1 cure. However, most of the currently explored antibody-based therapies and eradication strategies are based on neutralizing antibodies, especially those that are broadly neutralizing. By contrast, strategies for a functional cure with the use of non-neutralizing antibodies that eliminate HIV-infected cells through Fc–mediated effector function is largely unexploited avenue of research. Our program is designed to develop antibody conjugates of anti-HIV antibodies capable

of the most potent and broad Fc-mediated effector function that will be capable of sensitizing and killing HIV-1-infected cells from HIV-1-infected individuals trough Fc-mediated mechanism.

Marcelo Ramalho-Ortigao, D. Sc., Associate Professor
Department of Preventive Medicine and Biostatistics
Research Description: My overarching interests are on the biology of disease vectors, and research in my laboratory encompasses several aspects of the biology and physiology of disease vectors. Special focus in given to immunobiology of vector borne-diseases, transmission blocking methodologies, vector control, and sand flies and mosquitoes physiology, For transmission blocking, our primary model for investigation relies on sand fly transmission of Leishmania parasites. Our studies have led to the characterization of several sand fly molecules directly associated with vector-parasite molecular interactions, and the ability of Leishmania to develop within the sand fly vector. We have been involved in the characterization of sand fly genes regulated by Leishmania and identification targets for transmission blocking vaccines. With regards to immunobiology of vector borne-diseases and insect innate immunity, our efforts involve saliva variability and impact of disease transmission and distribution, and possibly as a tool in vector-biting assessment or prevention strategies. For vector control, we are currently focusing on developing larvicides for mosquito larvae control that are based on encapsulated essential oils, and on bio-control approaches for sand fly larvae that are associated with our studies of larval innate responses. Another area of investigation is paratransgenesis, or the use of modified commensal bacteria to interfere with pathogen development within the vector. A variety of tools are used in the laboratory, including cell/parasite culture, RNA interference, antibody and scFV expression, protein purification, cloning, insect rearing, infection/transmission studies. There is also a field component associated with investigations of vector populations, surveillance, and assessments of vector control tools. Many of the studies are done in collaborations with investigators the University of New Mexico Medical School, and overseas in such countries as Brazil and Tunisia.

Brian C. Schaefer, Ph.D., Professor
Department of Microbiology and Immunology
Research Description: Molecular mechanisms that control cellular immune responses. A major emphasis in my lab has been defining cytoplasmic signaling events that regulate leukocyte activation and proliferation, with a particular focus on T cell receptor (TCR) activation of the transcription factor, NF-κB. For this work, our experimental approach involves combining cutting-edge microscopy techniques with biochemical and genetic approaches to study the relationship between dynamic subcellular organization of signaling complexes and NF-κB activation. We are also investigating helpful and harmful immune responses in the central nervous system, previously in the context of traumatic brain injury and more recently in the context of neurotropic viral infections. In a collaborative effort with Dr. Broder's group, we have generated a new *in vivo* model to longitudinally trace neurotropic viral infections. We are using this system to develop novel therapeutics for such infections, defining how the host immune response can be redirected to successfully combat this class of viral pathogens. A third area of interest involves the biology of myeloid cells, including the investigation of myeloid cell biology in cancer and elucidation of novel molecular mechanisms regulating mitochondrial function in macrophages.

David W. Scott, Ph.D., Vice Chair for Research and Professor of Medicine
Department of Medicine
Research Description: Our laboratory focuses on immunologic tolerance research, including a more recent area involving gene therapy and the role of regulatory T cells in tolerance. Research has been aimed at translating novel therapies for a variety of diseases, including the modulation of autoimmune diseases (MS, T1D) as well as prevention and reversal of inhibitory antibody formation in hemophilia. The basis of our approach has been the use of chimeric proteins comprised of an immunoglobulin (Ig) heavy chain and the target domains or peptides. This has led recently to the discovery of putative regulatory

epitopes in *both* IgG and infectious agents that could turn on regulatory T cells. Our lab continues to be actively involved in the mechanisms by which T regulatory cells can modulate both innate and adaptive immune responses, *including adverse responses to viruses,* and are generating expanded human T regulatory cells engineered to express T cell receptors recognizing specific antigen.

Frank Shewmaker, Ph.D., Associate Professor
Department of Pharmacology
Research Description: Prions represent a unique class of infectious agents because they are composed of proteins and do not require a nucleic acid component for their infectivity. They are quite simply, infectious proteins. Prions are particularly notorious for being the infectious agents responsible for incurable diseases such as Creutzfeldt Jakob disease and Bovine Spongiform Encephalopathy (Mad Cow disease). Our laboratory studies prions of the eukaryotic model organism *Saccharomyces cerevisiae.* Several prions have been characterized in *Saccharomyces* and because they are not infectious to people, they offer an easy and safe way to study the fundamentals of prion propagation and transmission. The prion proteins we study form self-propagating amyloid structures when they are in their infectious forms. Amyloid is a highly-ordered protein aggregate with filamentous morphology that is often associated with neurodegenerative disorders like Alzheimer's and Parkinson's diseases. As a consequence, many of the fundamental aspects of the prions that we study have important parallels with amyloid diseases. Our laboratory is pursuing questions relating to amyloid structure and how it relates to prion formation, infectivity and propagation.

Clifford Snapper, M.D., Professor
Department of Pathology
Research Description: The Snapper laboratory is currently focused on developing prophylactic vaccines against Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV) suitable for clinical use. The vaccine components consist of multimeric recombinant viral envelope proteins expressed in Chinese hamster ovary cells, which induce virus-neutralizing antibodies in rabbits or mice in vivo. Further testing of EBV and HCMV vaccines in preventing viral infection and pathology in vivo will utilize humanized mice. The laboratory is also embarking on utilizing confocal microscopy to perform highly multiplexed quantitative 3-D analysis of immune responses in draining mouse lymph nodes following subcutaneous vaccinations with various adjuvants and distinct antigenic forms.

Andrew L. Snow, Ph.D., Associate Professor
Department of Pharmacology & Molecular Therapeutics
Research Description: My laboratory investigates the molecular mechanisms that control immune homeostasis in humans. More specifically, we study how inherited genetic mutations perturb signal transduction, differentiation, and apoptosis of lymphocytes in patients with immunodeficiency or immune dysregulation. Projects fall under two major themes. First, we are defining genetic, metabolic, and biochemical factors that govern apoptosis sensitivity in different subsets of human T cells. For example, we found that impaired T cell receptor restimulation-induced cell death (RICD) contributes to excessive lymphocyte accumulation and severe immunopathology in patients with X-linked lymphoproliferative disease (XLP). We are currently focused on delineating how SAP (the protein adaptor mutated in XLP patients), SLAM family receptors, co-inhibitory molecules (e.g. PD-1), and FOXP3 function as critical regulators of TCR-induced signaling and RICD. Second, we are discovering and characterizing novel human immune disorders linked to mutations in CARD11, a critical scaffold molecule required for antigen receptor signaling in T and B cells. We have shown that gain-of-function CARD11 mutations cause a selective B cell lymphoproliferative syndrome known as BENTA (B cell Expansion with NF-kB and T cell Anergy), but loss-of-function/dominant negative CARD11 mutations give rise to severe atopic disease. Interestingly, patients from both categories share features of immunodeficiency, including more frequent bacterial and viral infections, poor antibody responses, and impaired T cell function. In collaboration with both basic and clinical immunologists, we are characterizing a broad phenotypic spectrum of disease linked

to numerous unique CARD11 variants in >80 patients, and determining how these specific CARD11 mutations alter signaling and functions downstream of antigen receptor engagement. Ultimately, we hope to illuminate new therapeutic avenues for restoring lymphocyte homeostasis in myriad immune disorders by recalibrating proper signals for cell differentiation and death.

Ann Stewart, Ph.D., Professor
Department of Preventive Medicine and Biometrics
Research Description: Current research is focused primarily on understanding the impact of pre-clinical HIV infection on malaria transmission dynamics in an area of Kenya where both diseases are highly prevalent. In addition, we are using techniques of molecular genetics to evaluate drug and diagnostics resistance in reservoir populations of parasites in both Africa and South America. We are also involved in developing new technologies for malaria diagnosis in the field and in the laboratory.

Charles S. Via, 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 co-stimulatory 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 down-regulatory 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 down-regulating 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.

Kim C. Williamson, Ph.D., Professor
Department of Microbiology and Immunology
Research Description: Developing strategies to effectively block the spread of malaria is the long term goal of my research program. The need for a comprehensive approach has led to the development of a range of projects including 1) a systems biology project to understand the maturation of the immune response following repetitive controlled human malaria infections 2) molecular analysis of parasite differentiation and 3) anti-malaria drug development. The first project will use blood transcriptomics, immunophenotyping and antibody repertoire analysis to monitor the maturation of the immune response following repeat parasite exposure. The results will be correlated with clinical parameters, such as parasitemia and fever, to identify immune responses that contribute to protection. These insights will be

used to inform vaccine design. The second project seeks to understand the factors that regulate the growth and transmission of the parasite. In vitro work identifying the genes involved using forward and reverse genetic approaches are then utilized to monitor the process in the field. Similar molecular techniques are also being used to identify the mechanisms of action and targets of compounds found in a recent screen to have potent, multi-stage anti-malarial activity. Together these projects are designed to advance our understanding of host-microbe interactions and provide new tools to prevent infectious diseases.