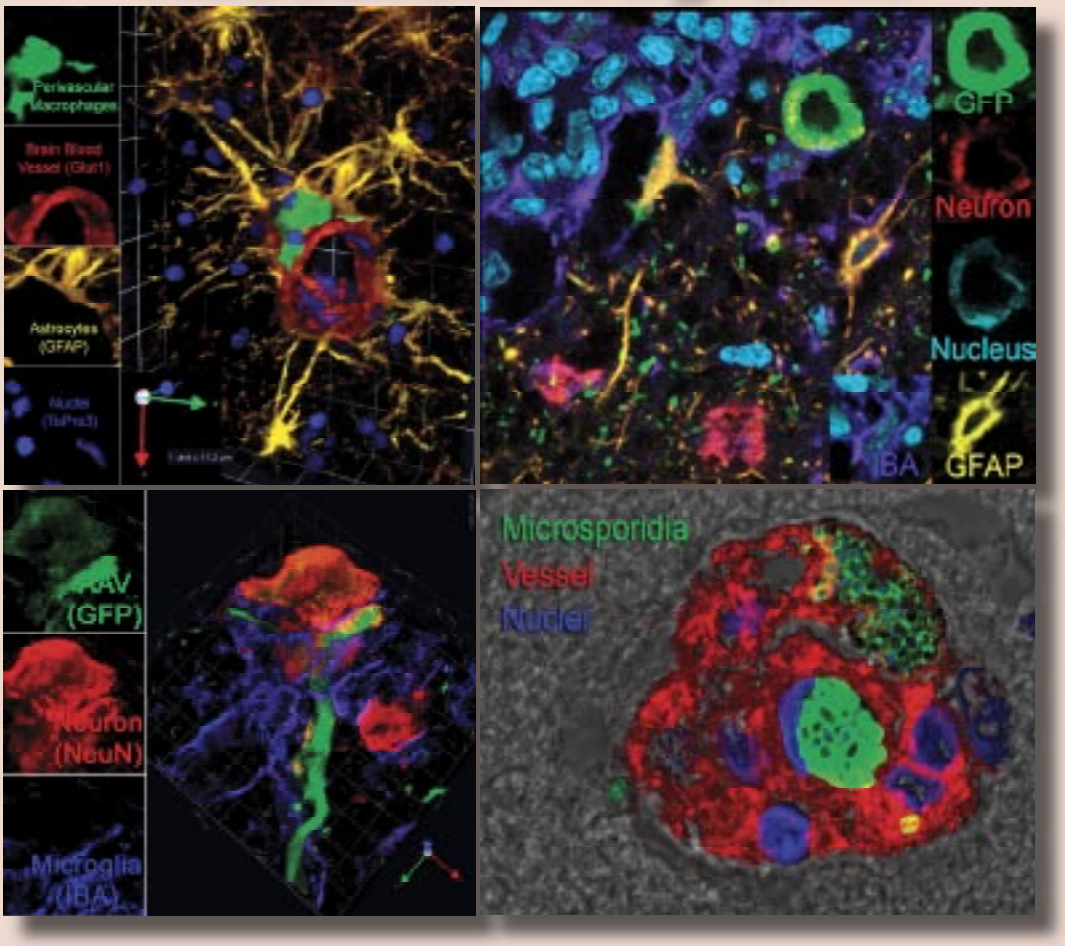
Tulane National Primate Research Center

Research Programs



Director

Andrew A. Lackner, DVM, PhD

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The Tulane National Primate Research Center is involved in a variety of important multidisciplinary projects focusing on areas of biomedical research with high priority concerns for human health. This brochure, which is intended for the interested researcher, scientist or investigator, provides information on those research programs and projects. The functions of each research program and project are discussed within the parameters of each division's research, research resources and educational and training opportunities. You will note that many of the Research Programs are collaborative involving more than a single research division.

Division of Bacteriology & Parasitology

The Division of Bacteriology & Parasitology encompasses research, training and service on features of the pathogenesis, diagnosis, treatment, transmission, and prevention of bacterial and parasitic diseases.

Research

Research in the Division currently centers on five diseases: Lyme disease, malaria, pneumonia caused by *Streptococcus pneumoniae*, tuberculosis, and brucellosis. Personnel devoted to research and its ancillary functions include five faculty, four postdoctoral fellows, one research scientist, two graduate students, six research technicians, and one administrative staff. Dr. Mario T. Philipp, Professor of Microbiology and Immunology at Tulane Medical School, is Chairman of the Division.

Lyme disease

The most extensive divisional program is on Lyme disease, or Lyme borreliosis. An emerging infectious disease that affects people in North America, Europe, and Asia, Lyme borreliosis is caused by the spirochete *Borrelia burgdorferi* and is transmitted by ticks. The disease may manifest in numerous ways, e.g. as an inflammation of the skin (erythema migrans), as arthritis, and in the peripheral and central nervous systems as, for example, facial paralysis and through neurocognitive symptoms. Lyme disease that affects the nervous system is also called Lyme neuroborreliosis. After developing a nonhuman primate model of Lyme disease, divisional faculty are currently using this model to try to understand the pathogenesis of neuroborreliosis of the central nervous system.

Improvement of methods for the serological diagnosis of Lyme disease is also of major interest to divisional faculty. The significance of this line of research stems from the fact that Lyme disease that is accurately diagnosed early in the course of infection is more efficaciously treated with antibiotics than when the disease remains undiagnosed for extended periods. The C6 test, an antibody detection assay that is more sensitive and specific than the tests available heretofore, was developed by investigators in the Division. An additional advantage is that the test does not yield false-positive results with serum specimens from humans or dogs that have received Lyme disease vaccination. The test was approved by the FDA and the USDA for human and animal use, respectively, and licensed by Tulane University to Immunetics, Inc., of Cambridge, MA, for human use, and to IDEXX Laboratories, Inc. of Westbrook, ME, for veterinary purposes. Currently research on diagnosis is focused on the C6 test as a predictor of Lyme disease therapy outcome, a possible new application of the test.

The spirochete *B. burgdorferi* has evolved numerous mechanisms of adaptation to which it must resort in order to survive in different host organs, in environments as dissimilar as ticks and mice, and in the face of a swift and intense immune response. Divisional scientists are researching the mechanisms of gene regulation that underpin these survival stratagems. Important findings have been made in relation to spirochetal adaptation to different cell densities and to changes in environmental pH and temperature, as well as relating to the ability of this organism to circumvent the antibody response of the host.

Malaria

Malaria, a mosquito-borne disease, is a major global health concern. The development of safe and effective anti-malarials is a worldwide priority, especially as treatment with chloroquine, the drug of choice for malaria for over 50 years, has been compromised by the increasing prevalence of chloroquine-resistant malaria parasites worldwide. A long-standing collaboration between investigators of the Division and the Department of Tropical Medicine and Parasitology of the Tulane School of Public Health and Tropical Medicine is devoted to developing new drugs that may be active against *Plasmodium falciparum* malaria strains that are resistant to chloroquine.

A basic study of key features of the host response to infection with another species that causes malaria, namely, *Plasmodium vivax*, also is being investigated through this same collaboration. Using human DNA microarrays and peripheral-blood mononuclear cells obtained from rhesus macaques that were infected with a simile of *P. vivax*, the monkey parasite *P. cynomolgi*, divisional investigators are studying transcriptome profiles of host gene expression during infection.

Pulmonary diseases: tuberculosis and pneumonia

The Division is in the process of expanding its research areas by attracting new faculty to work on the pathogenesis and immunoprophylaxis of tuberculosis and with new research initiatives in the area of pathogenesis of pneumonia due to *Streptococcus pneumoniae*.

Tuberculosis

Tuberculosis (TB), along with AIDS and malaria, is one of the three major infectious disease killers. One third of the world's population is infected with *Mycobacterium tuberculosis*. Each year an estimated eight million people develop, and about two million people die of, TB. Understanding bacterial gene expression *in vivo* is central to unraveling mechanisms of disease caused by intracellular bacteria such as *M. tuberculosis*. Using microarray technology, our faculty is comparing gene-expression patterns of the TB wild-type organism with the expression patterns of *M. tuberculosis* that are deficient in certain regulatory genes. It is hypothesized that these regulatory genes play a crucial role in the process of adaptation of M. tuberculosis to the intracellular milieu, and hold the secret of this bacterium's virulence.

Pneumonia

Infections caused by *S. pneumoniae* are a major cause of mortality throughout the world. There is a need to develop new and improved vaccines to prevent infection with this pathogen, due to limitations in the existing vaccines and as a response to increasing antibiotic resistance among *S. pneumoniae* isolates. In collaboration with scientists from the Louisiana State University and GlaxoSmithKline, investigators in the Division have

shown that the clinical course of disease caused by intra-bronchial inoculation of *S. pneumoniae* in normal adult rhesus macaques mimics aspects of the clinical course of human pneumococcal pneumonia. These findings, combined with the known fidelity with which this nonhuman primate species emulates the human immune response, underscore the utility of this model to assess the safety, immunogenicity and efficacy of newly developed *S. pneumoniae* vaccines. The model also may be useful to study innate and early acquired immune responses to *S. pneumoniae* as they occur in the lung and the specific aspects of pulmonary disease pathogenesis that pertain to infection with this common pathogen.

Brucellosis

Human brucellosis is a zoonotic infection caused by bacteria of the genus Brucella. Brucellosis remains endemic in many developing countries, where it undermines animal health and productivity and causes important economic losses. This disease also takes a toll in human health, with an estimated global incidence of 500,000 per year. The disease in humans is characterized by a plethora of symptoms, including fever, sweats, anorexia, fatigue, malaise, weight loss, and depression. The organism invades multiple organ systems, including cardiovascular, gastrointestinal, genitourinary, hepatobiliary, osteoarticular, pulmonary and nervous systems. Inflammation is a hallmark of brucellosis and may be a major contributor to disease pathogenesis. In collaboration with investigators from the University of Buenos Aires, Argentina, scientists from the Division of Bacteriology & Parasitology determined that lipoproteins, not lipopolysaccharide, are the key mediators of the pro-inflammatory response elicited by Brucella.

Research Resources

The Division has a significant service commitment to the Center. These service functions can be divided into four major areas: 1) Diagnostic Parasitology Core, 2) Vector-borne Diseases Core, 3) DNA Microarray and Expression Core, and, 4) Enzootic Pathogens Survey.

Diagnostic Parasitology

This Core provides diagnostic support to investigators and clinical veterinarians whenever monkeys are suspected of harboring parasites. All animals entering quarantine from outside sources are examined for blood and intestinal parasites at monthly intervals before they are allowed to enter the colony. Additionally, mice in the rodent colony are periodically checked for mites and pinworms prospectively. The Core also collaborates with researchers outside the TNPRC when projects involving nonhuman primates require diagnostic services. In addition, the laboratory is examining blood and fecal samples in direct support of a survey of enzootic pathogens from the breeding colony.

Vector-borne Diseases

This Core maintains insects and arthropods that are important to reproduce the natural mode of transmission of vector-borne diseases that are studied at the TNPRC, e.g. Lyme disease and malaria.

The DNA Microarray and Expression

This core provides both bioinformatics expertise to investigators who already have obtained DNA microarray results, and technical support to perform the microarray experiments de novo.

The Enzootic Pathogens Survey

This survey centers on the collection and examination of blood and stool samples from each animal in the TNPRC breeding colony. This survey permits the early detection of microbes that may affect the general health status of the monkey colony.

Education and Training Opportunities

The Division of Bacteriology & Parasitology is keenly aware that one of the four main components of the mission of the TNPRC is to provide training for postdoctoral fellows, graduate students, undergraduates, and visiting scientists. The appropriate training of scientists and veterinarians specialized in nonhuman primate research is of strategic importance to the future of our Center and that of the National Primate Research Center Program as a whole. Nowhere can such training be better accomplished than at the Centers themselves.

The Division has a long-standing track record of training post-doctoral fellows and graduate students. All past divisional fellows have successfully obtained further employment once their tenure at the Center ended. Types of employment that were sought and obtained included junior faculty positions, research scientist appointments both in governmental organizations and in industry, and senior postdoctoral fellowships. In the last five years, the Division trained five fellows. Three are currently on board.

In the main, divisional faculty members have served as advisors to graduate students from the Department of Parasitology of the Tulane Graduate School and from the Tulane Interdisciplinary Program in Molecular and Cellular Biology (MCBP). The Center has participated in the MCBP since the program's inception, and a faculty member has continually served on the MCBP Steering Committee. The chair of the Division of Bacteriology & Parasitology has been, until recently, the TNPRC representative to the MCBP Steering Committee and is now the Center's representative to the newly created, all-encompassing Graduate Program in Biomedical Sciences. Over the last five years, the Division has hosted three graduate students. Two are currently in training.

Capstone students (five in the last five years) from the Master in Public Health program of the Tulane School of Public Health and Tropical Medicine and undergraduate students (two in the last five years) from universities throughout the U.S. also have trained in the Division. The undergraduate students are part of the TNPRC Summer Student Training Program.

Visiting scientists from around the world have spent weeks to months training in the Division of Bacteriology & Parasitology. Most recently, two scientists worked on the immunology of Lyme disease and brucellosis. These scientists were, from the Central Drug Research Institute, Lucknow, India, and the University of Buenos Aires, Argentina.

In addition to their specific research program, all divisional trainees participate in the weekly divisional lab meeting and biweekly Journal Club. At the lab meeting, both practical and theoretical issues that arise during the week are discussed. The Journal Club focuses on molecular and immunological aspects of the host-microbe interface in bacterial infections. At the Center level there are, in addition, two colloquia: a monthly seminar on infectious diseases, with speakers invited from throughout the U.S., and a biweekly seminar, where faculty, post-docs, and graduate students present the work they realized in the previous six months.

Division of Collaborative Research

The Division of Collaborative Research facilitates the investigations of scientists outside the Tulane National Primate Research Center (TNPRC) with the resources at the TNPRC.

The Division consists of five full time members and two part time members including two faculty, three research technicians and two administrative staff. Dr. James L. Blanchard, Executive Director of Tulane's Comparative Medicine Program, is Chairman of the Division. Dr. Pyone Pyone Aye is the Division's Study Coordinator and also oversees the TNPRC's Pilot Study Program. If you are interested in more information about the Pilot Study Program, please see our web page at www.tnprc.tulane.edu.

The functions of the Division can be divided into two main areas, Research and Research Resources.

Research

Most of the research within the Division is collaborative with the Principal Investigator at a distant site. Below are examples of such projects.

Role of the Thymus in T Cell Homeostasis

By monitoring the changes in phenotypic T cell markers as well as in the numbers of T-cell Receptor (TCR) excisional circles – a recently described marker for recent thymic emigrants – following thymectomy, we have found evidence that surgical thymectomy in juvenile macaques results in a faster decay of peripheral CD4+ T-cells, but does not cause a substantial shift in CD45RA+ (naive) and CD45RA- (memory) populations. No compensatory extra-thymic source was detected in lymphoid tissues, although there was a small compensatory increase in T cell proliferation in the peripheral T cell pool. After SIV infection, thymectomized animals did not have higher viral loads, greater T-cell decay, or faster disease progression. We therefore conclude that peripheral destructive processes, rather than a loss of thymic output, appear to be the main causes of T cell depletion in SIV infection. Aaron Diamond AIDS Research Center.

Microbicides to Prevent AIDS Transmission

Cellulose acetate phthalate (CAP), a pharmaceutical excipient designed as a coating material for tablets or granules, has been demonstrated to be effective against herpes simplex virus type 2 (HSV-2) infection in mice, and to protect four of six rhesus monkeys from vaginal challenge with simian immunodeficiency virus SIVmac251. To assess whether CAP confers protection against primary viral strains that are transmitted in humans, infections with pathogenic simian/human immunodeficiency viruses (SHIVs) expressing the envelopes of X4 and R5HIV-1 strains (SHIVSF33A and SHSIVSF162P3, respectively) were performed. Seven of ten CAP treated macaques were protected from challenge with a mixture of X4-SHIVSF33A and R5-SHIVSF162P3. These findings in macaques suggest that CAP is efficacious against both X4 and R5 SHIV viruses *in vivo*, and should therefore be considered as a viable topical microbicide candidate in the prevention of HIV-1 infection. Aaron Diamond AIDS Research Center.

*SIV Epitope Specific CTL Responses in MAMU-A*01A*02 Double-positive Macaques*

A vaccine-elicited cytotoxic T-cell lymphocyte (CTL) response with a maximal breadth of epitope specificities should mediate the most efficient control of HIV-1 replication following infection. We studied the evolution of epitope-specific immune responses in naïve rhesus monkeys following infection with SIVmac251. The delay in the peak Mamu-A*02/pl99RY-specific CTL response in the presence of highly immunodominant Mamu-A*01-restricted CTL responses is most likely due to immunodomination, and represents the first demonstration of immunodomination in outbred primate species. Beth Israel Deaconess Medical Hospital, Harvard Medical School.

AT-2 SIV Vaccination after Dendritic Cell Mobilization

While both immature and mature monocytes-derived dendritic cells capture and present AT-2SIV to primed T cells *in vitro*, mature dendritic cells activate both CD4 and CD8 T cells but immature dendritic cells predominantly activated CD4 T cells. This supports the rationale for targeting activated dendritic cells with antigen for induction of the most effective immunity. Peripheral dendritic cells and mucosal dendritic cells respond to various stimuli comparable to human dendritic cells and can be mobilized *in vivo* following treatment with Flt3L. As little as 7 days treatment significantly increases dendritic cell numbers. The peak of the increase in dendritic cell numbers post treatment is under investigation. A study applying AT-2 SIV to the tonsils of naïve macaques revealed that SIV-specific T cells are primed and that pretreatment with immuno-stimulatory oligodeoxynucleotides (ISS-ODNs that activate peripheral dendritic cells), tends to increase the responses seen. Population Council, New York.

Implications for Viral Myocarditis

Coxsackie Virus B and Adenoviruses are the most common viral pathogens implicated in the development of human myocarditis. Both viruses gain entry into cells via the Coxsackie Adenovirus Receptor (CAR). We hypothesize that age-dependent CAR expression in cardiac myocytes may help explain the greater severity and higher mortality of coxsackie B (CVB) myocarditis in children, compared to adults. These data suggest that myocarditis and other manifestations of CVB infection in newborns may reflect higher CAR expression. To gain additional data, a macaque model of CVB myocarditis is being developed. UCLA School of Medicine.

Research Resources

Research resources provided by the Division are varied, but focus on providing outside investigators with the support needed to perform a research study at the TNPRC. This includes assistance with experiment design, budget preparation, regulatory paperwork, and sample acquisition, processing and shipping. In addition, the Division maintains a database on researchers assisted and quantifies the use of TNPRC resources being used to meet the needs of these investigators.

In the most recent reporting year, more than 11,000 tissue and blood samples were shipped to 61 outside collaborators in 22 states and 2 foreign countries. The Division also processed and shipped over 23,000 samples for diagnostic testing. Budget requests from over 30 core and non-core investigators were processed, resulting in 83 budgets totaling more than $56 million in animal related work. The Division also maintains a database of TNPRC collaborators totaling 489 investigators from 38 states and 22 countries. Monitoring

these types of activities results in accurate documentation of the TNPRC's role in supporting NIH-funded investigators throughout the U.S. In fact, in recent years, it has been shown that the TNPRC collaborative efforts have supported over $200 million in NIH funded research.

Division of Comparative Pathology

The Division of Comparative Pathology conducts tissue-based studies of disease processes with the major focus on infectious diseases including AIDS, malaria and tuberculosis.

The Division consists of thirty-eight members including nine faculty, five postdoctoral fellows, twenty-two research technicians, and two administrative staff. Dr. Ronald S. Veazey, Professor of Pathology at Tulane Medical School, is Chairman of the Division.

Research

The research component of the Division is very active and involves collaborative and independent work in a number of areas focusing on mechanisms of disease and development of new animal models. The research activities of the Division can be divided into investigations into the pathogenesis, treatment and prevention of AIDS, the effects of malaria on the immune system in pregnancy and children, and the pathogenesis and immunology of tuberculosis infections.

Acquired immune deficiency syndrome (AIDS)

More people die from AIDS each year than from any other infectious disease. Over 5 million people die from AIDS every year (about 14,000 per day) making this perhaps the greatest infectious disease threat to the human race. In the United States, AIDS is the second leading cause of death in young adults (18-40 years of age) and is second only to automobile accidents. Investigation of AIDS pathogenesis, prevention and treatment represent the largest research objective of the Division and encompass a number of projects examining 1) the macaque model of neuroAIDS, 2) AIDS and the mucosal immune system, 3) the development of microbicides to prevent HIV heterosexual transmission of AIDS, and 4) testing new therapies for AIDS.

Macaque model of neuroAIDS

Infection of rhesus macaques with SIV results in rapid neuroinvasion and neuropathologic abnormalities that are similar to those observed in HIV-infected humans. We have used this model to examine the role of chemokines and their receptors in neuroinvasion and the development of neuronal injury and neurologic disease. We previously demonstrated elevated expression of the chemokines MIP-1α, MIP-1β, and RANTES and the corresponding receptors CCR3, CCR5, and also CXCR4 in perivascular infiltrates in the brain. Of additional interest was the presence of CCR3, CCR5, and CXCR4 on a subpopulation of large hippocampal and neocortical pyramidal neurons and on glial cells in both normal and SIV-infected brain. The expression of known HIV/SIV coreceptors on neurons and astrocytes suggested a possible mechanism whereby HIV/SIV or the chemokines they induce could interact with these cells, disrupting their normal physiologic function and contributing to the neuropathogenesis of AIDS. To address this hypothesis, we have used immediately *ex vivo* and *in vitro* techniques to confirm the presence of all three of these chemokine receptors on a subpopulation of neurons and CCR5 and CXCR4 expression on the majority of astrocytes. These chemokine receptors are functional as demonstrated by increased intracellular calcium in response to the appropriate ligand. We have also used proton magnetic resonance spectroscopy (MRS) to evaluate the neuronal marker n-acetylaspartate (NAA) both *in vivo* and *ex vivo*. A 25% decrease in NAA was detected 14 days after infection coincident with peak viremia, neuroinvasion and increased numbers of perivascular macrophages. Decreases in NAA were correlated with decreases in synaptophysin and calbindin in adjacent sections of brain. A further 10%

decrease in NAA levels was observed in animals infected for two years or more regardless of the presence of SIV-encephalitis (SIVE). These results indicate that neuronal injury occurs early after viral infection, is associated with neuroinvasion and progresses during the course of infection. The interaction of chemokines or viral envelope with these functional chemokine receptors on neurons and astrocytes suggests a physiologic mechanism whereby neuronal injury could occur.

AIDS and the mucosal immune system

The Division has several ongoing AIDS-related projects to examine the pathogenesis, prevention, and treatment of HIV infection. Much of this work focuses on the vaginal and intestinal mucosa as well as the mucosal immune system in general. Previously, we had shown that rapid and profound CD4+ T cell depletion occurs almost exclusively within the intestinal tract of simian immunodeficiency virus (SIV)-infected macaques within days of infection. In more recent work, we have shown that intestinal CD4+ T cells have much higher expression of CCR5 than T cells in peripheral blood or lymph nodes. Furthermore, the selectivity and extent of CD4+ T cell loss may depend upon these cells co-expressing CCR5 and having a "memory" phenotype. These seminal studies we originally performed in macaques were finally confirmed in HIV-infected humans in 2004 which has resulted in a heightened interest in studying the mucosal immune system and AIDS.

In addition, we have also demonstrated that CCR5 expression on CD4+ T cells may be fundamentally involved in the pathogenesis of AIDS and disease progression (or the lack thereof) by examining expression of these markers in sooty mangabeys (SM) and African green monkeys (AGM). Sooty mangabeys and AGM are the natural host of SIV, and despite persistent high viral loads, these animals rarely progress to AIDS. We have recently discovered that both sooty mangabeys and AGMs naturally have markedly lower percentages of CD4+CCR5+ T cells in their tissues, which could explain why these animals do not progress to AIDS. This finding could have significance for treatment and vaccine strategies in HIV patients.

Heterosexual transmission of AIDS: Development of microbicides to prevent transmission

The development of a microbicide that could be applied to the vagina and prevent the transmission of HIV-1 could save millions of lives. Unfortunately, compounds that destroy HIV-1 are also likely to damage mucosal tissues. Alternatively, fusion inhibitors that attach to viral or host cell receptors may provide a safe and effective mechanism to prevent HIV-1 infection. In 2003, we demonstrated that applying a compound to the vagina of monkeys that blocked the molecule responsible for viral attachment to the host CD4 molecule could completely prevent the vaginal transmission of SHIV (a virus having the HIV outer envelope). In 2004, we demonstrated that a CCR5 blocking agent (PSC-RANTES) could also completely prevent the vaginal transmission of SHIV when introduced as a topical application in the vagina. These studies were the first to demonstrate that CD4 or CCR5 fusion inhibitors could be part of an effective HIV preventative (microbicide). In more recent studies, we have demonstrated that a small molecule inhibitor of CCR5 can prevent vaginal SDHIV transmission. We are continuing to test this fusion inhibitor with the goal of producing a cheap and effective microbicide that could be distributed to places where the epidemic is rampant to slow the spread of HIV infection.

Testing new therapies for AIDS

Although remarkable progress has been made in anti-viral therapies in the last few years, no patient has been cured of infection, and increasingly, patients are showing resistance to anti-HIV drugs in use today. With the rapid and spreading emergence of multi-drug resistant strains of HIV, new classes of anti-HIV therapies are needed to combat HIV infections and for the prevention of AIDS. We are currently testing fusion inhibitors

in SIV-infected rhesus macaques to determine whether they may affect viral loads and whether they may be useful as single or combinational therapies against SIV and SHIV-infected macaques. We have demonstrated that certain compounds have remarkable efficacy in reducing viral loads in SIV-infected macaques and are currently testing whether these changes correlate with reductions in chemokine receptor expression, and/or result in viral envelope mutations that may result in drug resistance. These compounds are being tested for efficacy, drug resistance and safety in nonhuman primates.

Malaria

The Division has developed a nonhuman primate model of malaria during pregnancy. *Plasmodium coatneyi* is used to infect rhesus monkeys with severe malaria during pregnancy and induces clinical features and placental pathology very similar to those caused by *P. falciparum* in pregnant women. The goal of these studies is to determine the etiology of the poor fetal outcome (abortion, low birth weight, early infant death, etc.) that occurs when pregnancy is complicated by malaria. The aims include the role that parity (number of prior pregnancies) and prior immunity to malaria plays, as well as the direct and indirect effects of malaria on the maternal and fetal immune system. Much of this work involves investigating the combination of immune responses associated with successful pregnancy, the effective defenses against malaria, and the effect that these two diametrically opposed responses have on the placenta and placental function. This work has led to collaborative studies of human placental pathology induced by *P. falciparum* and *P. vivax* in pregnant women at the Shoklo Malaria Research Unit in Mae Sot, Thailand.

Tuberculosis

Work on tuberculosis is focused on development of new vaccines and diagnostics. The vaccine work is a collaborative effort with investigators from Duke University, Albert Einstein College of Medicine, and Harvard University. In these studies, we are using gene deletion mutants of *Mycobacterium tuberculosis* developed by our collaborators and designated 6020 and 6030. These two deletion mutants are extremely safe in rodents and have been shown to be more effective at preventing tuberculosis (TB) than BCG. We have shown that these mutants are also safe in *cynomolgus macaques*, and we are in the middle of a protection study comparing 6020 and 6030 with BCG.

We are also working on testing and developing improved diagnostic tests for tuberculosis. A rapid test has been developed that is an easy-to-perform, single-use diagnostic test for visual detection of antibodies to the mycobacterium that cause tuberculosis. This format provides a test that is simple (requires neither electricity nor expensive equipment for test execution or reading, nor skilled personnel for test interpretation), rapid (turn around time less than 15 minutes), safe (minimizes handling of potentially infected specimens), non-invasive (requires 30 µl of serum), stable (can keep at least 18 months without refrigeration) and highly reproducible. We are currently testing the reliability of this kit in normal non-infected monkeys, false-positive skin test animals, and monkeys experimentally infected with tuberculosis to prove that it is specific, safe, and reliable for testing humans for the presence of the tuberculosis causing mycobacteria.

Research Resources

The Division has a significant service commitment to the Center, and these service functions can be divided into four major areas: 1) necropsy and biopsy service, 2) clinical pathology, 3) molecular pathology, and 4) confocal microscopy and image analysis.

Pathology service support is provided to the Center's clinical veterinary staff as part of our colony surveillance program. The Division also furnishes support in each of these areas to staff scientists within other divisions at the Center as well as to collaborating scientists from around the world.

Necropsy and Biopsy Service Core

The necropsy and biopsy service is the core of the Division's service functions. The necropsy and biopsy service provides investigators and collaborators at the Center with gross and histopathologic evaluations of organs and tissues for the purpose of understanding pathologic changes either for diseases that spontaneously arise in the colony or in relation to experimental protocols. In addition, the service assists the clinical veterinarians with colony health and management. The necropsy and biopsy service is the Center's primary means to identify and investigate new conditions in nonhuman primates. These investigations serve not only to provide further understanding of disease processes in general, but also have the potential to identify new models for the study of human disease.

Clinical Pathology Core

The Clinical Pathology Laboratory Core provides clinical data for animals involved in specific research projects as well as colony animals. The laboratory furnishes hematology, chemistry, fecal analysis, fluid analysis, urinalysis, and cytology on all Center animals. The Clinical Pathology Laboratory Core is currently staffed by two Medical Research Specialists and a Laboratory Supervisor, all three of whom are ASCP registered Medical Technologists. The primary responsibility of this unit is to perform hematology, clinical chemistry and bacteriology for the medical care of the animal colony and to support numerous research projects from scientists here at the TNPRC and around the world. The service also provides important diagnostic support for the veterinarians in managing the health and well being of the animals in the colony.

Molecular Pathology Core

The Molecular Pathology Core facility provides investigators at Tulane and around the world with an array of tools and techniques to investigate the molecular mechanisms involved in diseases. The Core facility provides multilabel immunohistochemistry, PCR testing, the generation of labeled RNA and DNA probes (for cytokines, EGFP, and SIV) via *in vitro* transcription or random primed labeling, *in situ* hybridization on paraffin and frozen tissue sections, and molecular manipulation of genetic and plasmid material to provide the necessary templates. This section of the Pathology Division also provides a significant amount of consulting regarding molecular techniques to scientists, technicians, and postdoctoral fellows in molecular biology techniques.

Confocal Microscopy and Image Analysis Core

This Core provides state-of-the-art confocal microscopy, multilabel fluorescent labeling and detection, and image analysis support to every Division in the TNPRC and to numerous affiliate research scientists at several institutions around the world. The Core has a Leica TCS SP2 laser scanning confocal microscope system equipped with three lasers, with six laser lines available, capable of simultaneously collecting information in four channels (three fluorescent and one for differential interference contrast). The system is attached to two microscopes, upright (DMRE) and inverted (DMIRE2), that allow for confocal microscopy of fixed preparations and also living cells. The confocal system offers such benefits as: a) multi-dimensional imaging, as it is possible to obtain images in four dimensions, length (x axis), width (y axis), depth (z axis) and time (t); b) resolution improvement; c) contrast improvement; and e) multicolor imaging. Several fluorochromes can be imaged at the same time.

Education and Training Opportunities

The Division is currently involved in a variety of training programs including preparatory training for the Board Certifying Examination of the American College of Veterinary Pathologists (ACVP). Two of our faculty are board-certified by the ACVP. The TNPRC is also an active and participating member of the Armed Forces Institute of Pathology (AFIP) Wednesday slide conference, in which we routinely receive and submit interesting case files for the purpose of teaching and training of veterinary pathologists. We also have an established research and veterinary pathology training program in conjunction with the Louisiana State University School of Veterinary Medicine in Baton Rouge (about an hour away) in which the faculty and students participate jointly in a variety of research and veterinary pathology training programs.

The Division also provides extensive research training in immunohistochemistry, confocal microscopy, flow cytometry, *in situ* hybridization and other molecular biology techniques for postdoctoral fellows and graduate students.

Division of Gene Therapy

The Division of Gene Therapy conducts regenerative medicine research that utilizes vector-based gene therapies and cell-based interventions for genetic and acquired diseases.

The Division consists of nine members including one faculty, four postdoctoral fellows, three research technicians, and one administrative staff. In addition, the Division has one graduate student. Dr. Bruce A. Bunnell, Associate Professor of Pharmacology at Tulane Medical School, is Chairman of the Division.

Research

The research component of the Division is active and involves collaborative and independent work in a number of areas. Studies focus on the development of gene and cell-based therapies for diseases of the central nervous and pulmonary systems.

The research activities of the Division can be divided into investigations of gene therapy, stem cell biology and therapeutic applications and characterization of a nonhuman primate model of Krabbe's disease (www.huntershope.org or www.ninds.nih.gov/disorders/krabbe).

Vector-based gene therapy is a central focus of the Division. This work involves efforts to develop vector-based gene therapy strategies for Krabbe's disease. The lab is currently developing a series of lentivirus vectors for the expression of GALC, the defective gene in Krabbe's disease, both *in vitro* and *in vivo*. Our group has also established collaborations with two investigators from the University of Pennsylvania. We are presently investigating the biodistribution and transduction patterns for novel adeno-associated virus (AAV) serotypes and serotypes of lentivirus vectors.

The stem cell program is presently focused on three sources of adult stem cells; mesenchymal stem cells (MSCs) from bone marrow and adipose tissue and neural stem cells directly from the brain. In the coming year, we will be collaborating with Don Wolf from the Oregon NPRC by obtaining rhesus embryonic stem cells and doing direct comparisons between these and adult stem cells in the CNS. With our connection to the Krabbe's Colony, we have begun to focus both of these research areas toward therapy for Krabbe's disease. The Division is investigating the application of stem cells for pulmonary diseases in collaboration with investigators from the University of Vermont and the University of Pittsburgh. Lastly, the Division is beginning exciting research that assesses the therapeutic benefits provided by various stem cell populations in a nonhuman primate model of stroke.

The Division is also responsible for coordinating studies in the only model of genetic disease in nonhuman primates, the Krabbe's monkey. As such, the Division is focused on the development and analysis of novel gene therapy strategies for lysosomal storage diseases. More importantly, the Krabbe's rhesus monkeys are an invaluable national resource that will be available to investigators nationwide.

Research Resources

The Division has a significant service commitment to the Center. These service functions can be divided into two major areas: 1) viral vectors and 2) mesenchymal stem cells.

Vector Development and Production Core (VDPC)

The VDPC directs the production of the oncoretrovirus, lentivirus, and recombinant AAV vectors. The virus vector production facility maximizes resource utilization and efficiency by centralizing the production of recombinant virus vector production so that it is all done using standard protocols and the same strict guidelines for all of the gene therapy projects that are pursued at TNPRC. By providing vector core services, the VDPC will have a large impact not only on the Gene Therapy Program but also on the Divisions of Comparative Pathology, Microbiology and Immunology at the TNPRC and on other departments and centers at the Tulane University Health Sciences Center.

The goals of the Core are:

1. Prepare recombinant virus vectors for use in gene therapy and vaccine studies.
2. Consult with investigators on vector design and construction.
3. Develop novel MLV, LV and AAV vectors for future core use.
4. Explore novel virus vector systems for use in core production.

Stem Cell Production Core (SCPC)

Mesenchymal stem cells (MSC) are a subset of adult stem cells from bone marrow or adipose tissue. These cells are of medical and therapeutic interest because they have been shown to differentiate into osteoblasts, adipocytes, chondrocytes, myocytes, astrocytes, oligodendrocytes and neurons. Due to their inherent plasticity, these cells have the potential to be useful for the treatment of a large number of genetic diseases. The Mesenchymal Stem Cell Production Core Facility (SCPC) focuses on generation, maintenance and distribution of nonhuman primate MSCs. We routinely prepare MSCs from rhesus macaque bone marrow and adipose tissue samples.

The goals of the Core are:

1. Prepare a continuous supply of quality-tested nonhuman primate MSCs for distribution to other investigators.
2. Develop improved methods for isolating and characterizing nonhuman primate MSCs.
3. Prepare MSCs engineered to express various reporter genes enhanced green fluorescent protein (EGFP), beta –galactosidase (β-gal), luciferase, etc.)
4. Consult with investigators on design of research studies using MSCs.

Education and Training Opportunities

Training opportunities are available in the Division for postdoctoral fellows, graduate students, and veterinary residents and fellows in the following areas: basic biology of bone marrow and adipose tissue derived mesenchymal stem cells, therapeutic intervention in Krabbe's disease, therapeutic applications of stem cells for lung diseases and stroke, and viral vector-mediated gene therapy for CNS diseases.

Division of Immunology

The Division of Immunology conducts basic and applied immunology studies of disease processes with a focus on infectious diseases such as AIDS, Lyme disease and Rickettsia.

The Division consists of eleven members including two faculty members, two postdoctoral fellows, six research technicians, and one administrative staff. Dr. Marcelo J. Kuroda, Associate Professor of Microbiology and Immunology at Tulane Medical School, is Chairman of the Division.

Research

The research component of the Division of Immunology is active and involves collaborative and independent work in a number of areas focusing on mechanisms of disease and development of new animal models.

A major current focus is on basic aspects of cellular immunology in the monkey model of AIDS.

We study virus-specific cellular immune responses specific for the AIDS virus in a nonhuman primate model of AIDS, the simian immunodeficiency virus-infected rhesus monkey. These studies are done using both functional assays and flow cytometry, employing tetrameric MHC class I or II/peptide complexes. These tetramers are used as staining reagents in flow cytometric analyses to quantitate and define the phenotype of antigen-specific T lymphocytes. Using these reagents, we were able to demonstrate a clear correlation between cytotoxic T cell (CTL) expansion and clearance of viral replication during primary SIV infection. Moreover, the distribution of virus-specific CD8+ T cells in various lymphoid compartments and their association with localized virus replication has also been assessed. The precise quantitative power of this technology has allowed us to compare different vaccination modalities in order to develop an effective vaccine against HIV infection. We have also succeeded in developing tetrameric MHC class II peptide complexes to quantitate antigen-specific CD4+ T cells in virus-infected and vaccinated rhesus monkeys. We have selected the rhesus MHC class II, Mamu-DR*W201 for the construction of the class II tetramers, because we found that this molecule is expressed in 35-40% of all rhesus monkeys tested from four different colonies. This new technology has facilitated the quantitative analysis of simian immunodeficiency virus (SIV)-specific T cell responses *in vivo* in monkeys following virus infection and immunization. To better understand the biology of the antigen–specific CD8+ and CD4+ T lymphocytes in SIV infection, we are currently extending the usage of the tetramer technology to other new MHC class I and II alleles.

Recently, to expand our ability to analyze the MHC-TCR interaction from the opposite perspective, we have constructed a TCR tetramer by reassembling α and β chains derived from a peptide-specific T cell population into a functional antigen-specific TCR. The TCR tetramer recognized the restricting MHC class I molecule only in the peptide-bound state and with high specificity and avidity. Importantly, subtle changes in cognate peptide levels bound to the class I molecule were accurately reflected by parallel changes in the mean fluorescence intensity of cells that bound the TCR tetramer. Furthermore, we showed that the TCR tetramer blocks expansion *in vitro* of CTL specific for the cognate peptide, but has no effect on the expansion of CTL specific for control peptides restricted by the same class I allele. The stringency of this tetramer for its restricting class I allele was also demonstrated by accurately identifying animals expressing the restricting Mamu-A*01 class I allele from a large cohort of over 100 outbred rhesus macaques. With its high throughput potential and reproducibility, TCR tetramer-based approaches can help explore the MHC/peptide interface in detail. With

the described tools in hand, we are currently using both mouse and rhesus macaque models to study the fate of memory as well as naïve antigen specific CD8+ and CD4+ T cells using dendritic cells as professional antigen presenting cells. Detailed knowledge of the interaction of APC with antigen specific T cells will be needed to design effective vaccine strategies as well as immune-based therapy to infectious diseases, tumors and autoimmune diseases.

Research Resources

The Division of Immunology has a significant service commitment to the Center. These service functions can be divided into two major cores: 1) Flow cytometry core, 2) Immunology core.

Flow Cytometry Core (FCC)

The FCC is equipped with two FACS-Calibur analytic flow cytometers capable of 4-color analysis, one FACS Aria high-speed cell sorter capable of 9-color analysis and simultaneous 4-way cell sorting and a LSRII analytic flow cytometer capable of 12-color analysis. The FCC offers assistance ranging from study design through data acquisition and analysis.

Immunology Core (IC)

The IC provides immunology services to specific research projects of in-house and outside investigators, as requested. Current services include sample preparation under standardized procedures for optimal assay analysis, planning and performance of ELISPOT assays, data processing and presentation, and intracellular cytokine staining.

Education and Training Opportunities

The Division offers training opportunities to postdoctoral fellows, undergraduate students, graduate students and research fellows in the area of AIDS immunology and vaccine development

Division of Microbiology

The Division of Microbiology conducts basic infectious disease research using nonhuman primate models of human disease with an emphasis on AIDS, microsporidial infections, vaccine development and retrovirology.

The Division consists of thirty-two members including eight faculty, four post-doctoral fellows, four visiting scientists, four administrative staff, ten research technicians and two graduate students. Dr. Preston A. Marx, Professor of Tropical Medicine at Tulane School of Public Health and Tropical Medicine, is Chairman of the Division.

Research

The functions of the Division consist of research, research resources and education and training.

The research activities of the Division can be divided into AIDS related and non-AIDS related. Research on AIDS includes the origins and genetic diversity of HIV, pathogenesis as it is related to progression, long term latency and cell biology, a nonhuman primate model for heterosexual transmission of HIV and vaccine development. Non-AIDS research can be grouped into projects involving, microsporidia and its associated diseases, human and simian T cell leukemia virus, varicella virus, respiratory syncitial virus, human and rhesus papilloma virus, and rotavirus. Brief descriptions of major research programs are provided below.

Origins and Genetic Diversity of HIV and the Emergence of Pandemic HIV

Research in the Division has focused on identifying SIV genetic lineages that are ancestral to HIV with the goal of understanding the genetic diversity of primate lentiviruses. Scientists in the Division have discovered new SIVs in western and equatorial Africa that are members of the HIV family tree. The ancestral viruses to HIV-1 and HIV-2 are SIV from the chimpanzee and the sooty mangabey, respectively. However, the SIV group infects over 40 monkey species in Africa. One focus is finding out if the other SIVs that infect other African monkey and ape species pose a risk for human infection and the emergence of new HIV strains and types. Another major effort is to understand the steps leading to the transition of SIV to HIV.

Pathogenesis of SIV

The SIV animal model is being used to conduct basic research on the immunopathogenesis of SIV infections. The research investigates cross species transmission of SIV, long term non progression and the absence of disease in African nonhuman primates naturally infected with SIV.

It is now clear that AIDS originated from cross species transmission of SIV from one or more species of African nonhuman primates to humans. Thus, a better understanding of cross species transmission, emergence, and adaptation of SIV to new human and nonhuman primate hosts is of major importance. The mechanisms by which SIV successfully adapts to new hosts will shed light on the origins of pandemic strains of HIV and will apply to other viruses as well. To address this question, SIV strains obtained directly from sooty mangabeys are being used to infect rhesus macaques to understand how SIV adapts to a new host species. The results of these studies will contribute to the understanding of how new epidemic strains of HIV evolved and emerged in the 20th century.

It is well known that HIV infection of humans is fatal in five to ten years if left untreated. However, a small percentage of HIV infected persons suppress the infection naturally and live for longer periods without symptoms of AIDS. Little is known about these elite long-term nonprogressors (LTNP), and it is difficult to study in humans, especially in the early stages of infection. Research in the Division has recently shown that a significant percentage of SIVmac-infected rhesus of Chinese origin become LTNP analgous to elite LTNP in humans. Research is ongoing to understand the dynamics of immune cell restoration and control of infection in these animals.

Several species of nonhuman primates native to Africa are naturally infected with SIV and rarely develop disease. Understanding how these animals can maintain a life-long infection without resulting in disease is likely to offer important clues to help develop strategies to prevent AIDS in humans. Toward this end, we are examining African green monkeys and sooty mangabeys. Each species is infected with its own strain of SIV, and each seems to have developed unique mechanisms to cope with the infection. Only two examples of AIDS are known in naturally infected sooty mangabeys and African green monkeys. Both studies were done in the TNPRC Division of Microbiology.

Heterosexual Models for HIV Transmission

The majority of HIV infections occur by heterosexual transmission. Investigators in the Division were the first to develop an animal model to study sexual transmission. Using SIVmac and female rhesus macaques, infection was readily transmitted across the intact vaginal lining. Infection became systemic and resulted in the development of AIDS. This model closely follows AIDS development in HIV-infected women and is being used to study prevention, treatment and pathogenesis. Recent studies in collaboration with the Division of Comparative Pathology have focused on monoclonal antibodies to prevent SIV vaginal infection. There is a critical need for inexpensive drugs that can prevent HIV transmission to women because vaccines are not yet ready for use. Drugs that can be used safely and discreetly by women to prevent HIV infection are of great interest, particularly in developing countries. The monoclonal antibody b12 had been used as a topical prophylactic in the vagina and shown to be effective at blocking infection. However, b12 is rare among antibodies in that it can neutralize HIV and SHIV (a simian human hybrid virus). In ongoing studies, mutants of b12 are being tested to understand the mechanism by which b12 antibody can prevent vaginal SHIV infection.

Other uses of the model are the study of the role of the female hormones, progesterone and estrogen, in SIV vaginal transmission. A long-term study has shown that progesterone increases the susceptibility of the vaginal lining to SIV infection. In contrast, estrogen prevents infection. Prevention correlated with an increase in thickness of the vaginal lining which is hypothesized to block infection. This finding can be exploited to develop new prevention strategies. The most recent development applies a vaginal cream containing estrial. Estrogen-based vaginal creams are routinely used by women to treat post-menopausal vaginal symptoms. Estrogen creams induce changes in the vaginal microenvironment that increase resistance to SIV infection. The results have been promising, and a new study is planned in women to examine the changes in the vagina that may promote resistance to HIV infection.

AIDS vaccines

AIDS vaccines are a major focus in the Division. Live vectors and immunogenic peptides have been used. The SIV macaque model is also used by Division collaborators having AIDS vaccine programs funded by NIH.

Live vaccine vectors have included vesicular stomatitis virus (VSV). VSV is relatively non-pathogenic in monkeys and humans. VSV-HIV hybrid viruses have been constructed to express HIV and SIV genes. The safety, immunogenicity and efficacy of these vectors expressing SIV genes have been tested in the SIV/macaque model of AIDS. Animals have been immunized by intramuscular, oral and intranasal routes to determine which induces the strongest and most durable immune response. The humoral and cell-mediated immune responses were measured. The appearance of antibodies was measured and compared between different VSV-hybrids and different routes of immunization. SHIV was used to challenge immunized macaque monkeys. The candidate vaccine induced strong protection in the SHIV model. The immunized monkeys were not observed to have any deleterious effects from the immunization protocol with the live vectors. Upon challenge with a pathogenic SHIV virus, the immunized monkeys suppressed the virus compared to the mock vaccine control group. Because of the success of this series of experiments,VSV-HIV hybrid vectors are being developed for clinical trials in humans. These new vectors for human use are currently being tested in rhesus macaques to determine if they are safe and immunogenic.

Another vaccine approach in the Division is the use of synthetic peptides derived from immunogenic epitopes of the gag and env gene of HIV and SIV. These peptides were incorporated into immunostimulating complexes known as ISCOMS. This and other novel approaches are being pursued in the Division.

Natural History of Microsporidial Infections and Therapeutic Approaches

Microsporidia are single-celled parasites associated with diarrhea and systemic disease in animals and humans worldwide. Horizontal, zoonotic, waterborne, and food borne modes of transmission occur, thereby resulting in targeted research on the microsporidia by the U.S. Environmental Protection Agency in response to the Safe Drinking Water Act and by the National Institutes of Health and Centers for Disease Control and Prevention in response to the potential bioterrorist threat of these organisms. Research on microsporidiosis in the Division focuses on infection of humans and nonhuman primates by species of microsporidia, namely *Enterocytozoon bieneusi* and three *Encephalitozoon* species. Studies are being conducted to improve diagnostic tests as well as to characterize the natural history and pathogenesis of these infections. These studies serve to better understand the course of microsporidial infections in humans. Effective therapies are lacking to treat all the microsporidia that infect humans and animals, but fumagillin and related compounds show great potential. Toxicity concerns, however, have led to studies to clone, express, and characterize methionine aminopeptidase 2, the putative fumagillin target of the microsporidia, and to develop a high throughput screening assay that can be applied to identify more effective and less toxic fumagillin analogues. Tissue culture and murine and nonhuman primate models of microsporidiosis are being used for preclinical testing to evaluate efficacy and toxicity of lead compounds. In addition, immune responses are being defined in these models to better understand the mechanisms by which T cells and macrophages interact to kill microsporidia. Finally, methods are being developed and applied to capture and identify microsporidia in water sources that pose a potential risk for transmission to humans and animals.

STLV and HTLV

Simian and human T cell leukemia virus and (STLV and HTLV) are important pathogens causing life-long chronic infections that may lead to T-cell leukemia/lymphoma (ATLL) and a variety of neuromuscular diseases of humans. An animal model was developed in the Division using STLV which is closely related to HTLV-1. STLV was associated with abnormal lymphoproliferation and produced hyperplastic lymph nodes that were clonal outgrowths of STLVagm-infected cells similar to those seen in humans with ATLL. In addition, there was immunosuppression and widespread dissemination of a naturally occurring simian immunodeficiency virus (SIVagm). We have several species-specific isolates of STLV and are characterizing them genetically, as well as for their ability to transform lymphoid cells *in vitro* and for their ability to experimentally infect and cause disease in the nonhuman primate.

Collaborative studies on HTLV include the experimental transmission of human clinical isolates of HTLV-I to nonhuman primates. In certain geographic regions, HTLV co-infection of HIV-I positive individuals has a prevalence of 5-10%, and is hypothesized to increase HTLV viral expression and the risk for HTLV-associated diseases. Macaques co-infected with SIV and STLV have served as a model for this human infection and disease.

Varicella Virus Infection, Latency, and Reactivation

The Varicella-zoster virus causes clinically mild chickenpox in children. However, in older or immunocompromised individuals, infection is generally more severe and may be life threatening. In addition, latency of the virus in dorsal root ganglia is common, and reactivation of the virus late in life is responsible for significant morbidity due to viral zoster, or shingles. A nonhuman primate model of infection and disease in the African green monkey was developed and is being used to explore these issues. Collaborative studies on immunomodulatory therapeutics for control of infection are also underway. In additional collaborations, chimeric varicella viruses are being tested to delineate genes responsible for varicella latency, and ultimately, control of reactivation. A candidate SIV vaccine using simian varicella virus vector is under development.

Respiratory Syncytial Virus Infection and Pathogenesis

Respiratory Syncytial virus (RSV) is a major cause of serious lower respiratory tract disease in infants and young children. Hospitalized children with underlying heart and lung conditions are at the most risk for severe complications. No effective vaccine is available, and the current antiviral drug available has limited use. A nonhuman primate model of RSV infection and disease has been developed and is being utilized to test various RSV vaccine candidates and therapeutics.

Rotavirus

A nonhuman primate model of human rotavirus infection has been developed using a new rotavirus strain isolated from nonhuman primates at the Tulane center. The virus was characterized as serotype G3, subgroup 1, genotype P and designated the Tulane University Cincinnati (Children's) Hospital (TUCH) strain. The rotavirus model using juvenile macaques is being used to develop new vaccines and to elucidate the mechanism(s) of how rotavirus evades the host innate response, and adaptive immunity to further characterize the correlates of rotavirus protection.

Research Resources

The Division of Microbiology maintains two service cores to support the clinical staff and investigators that use the resources of the TNPRC. These are:

Viral Diagnostic Core (VDC)

A healthy nonhuman primate (NHP) colony that is free of specific pathogens is vital to the success of a primate center. The VDC, established in 2003, is a key resource in this regard. It has two components:

1. The viral diagnostic component provides serological testing for the NHP colony. For the "Specific Pathogen Free" colony, each monkey is tested for SIV, STLV, SRV and herpes B virus infection. For SIV specific antibody detection, a peptide ELISA against SIVmac/SIVsm was developed. Confirmation of SIV antibody is provided by a Western blot (WB) assay. Herpes B virus serodiagnosis is done using a commercial Herpes simplex virus type1 ELISA. Commercial ELISA kits and a second generation WB assay that allows discrimination between STLV-1 and STLV-2 are being used for diagnosis of STLV infections. The methodology for SRV diagnosis is currently under development. The VDC processed approximately 3,000 samples in the past year and is growing to meet future needs.

2. A real-time PCR component is also part of the Viral Diagnostic Core. Currently SIVmac and SIVagm quantification is available. Other services, such as cytokine and chemokine quantification, are also available. Additionally, the Core will assist investigators with custom-designed real-time PCR assays.

The VDC also provides other serological assays upon the investigator's request. Examples are TB Primagam and SIV p27 antigenemia quantification.

Retrovirus Challenge Stock Production and SIV/SHIV Isolation Core

This Core provides research investigators with primate lentiviruses for inoculation of nonhuman primates (NHP). The Core fills this need because many investigators who use NHPs are not able to produce and maintain their own virus challenge stocks. Therefore, the Core produces and maintains titered viral stocks for testing the efficacy of candidate AIDS vaccines, for testing new anti-HIV drugs and for viral pathogenesis studies. The inventory consists of 65 separate stocks of SHIVs and SIVs. These viruses were grown in human cell lines and in primary cell cultures of human and NHP origin. The Core has also prepared specially requested stocks for use by investigators.

Education and Training Opportunities

Members of the Division are engaged in education and training at multiple levels including summer fellowships for undergraduates, graduate students and postdoctoral fellows. In addition, senior members of the faculty are heavily involved with mentoring junior faculty. Mentoring has resulted in new faculty obtaining significant external research support. The Division fully participates in Tulane graduate programs with Division faculty serving as major professors for these programs.

Division of Veterinary Medicine

The Division of Veterinary Medicine is responsible for all aspects of care, husbandry and management of the animal colonies. This responsibility includes the provision of clinical veterinary medical care, development and implementation of the nonhuman primate enrichment program, maintenance and development of the animal records database, research support, and collection of biological specimens.
The Division administers the Veterinary Resources program through six individual units that work in conjunction to accomplish Division objectives. The units are the Office of the Associate Director for Veterinary Resources and Chair of the Division, Clinical and Research Medicine, Research Resources, Animal Resources, Environmental Enrichment, and Reproductive Biology.

The Division consists of approximately one hundred members including nine full-time faculty, one laboratory animal medicine resident, veterinary and animal care technicians, and administrative staff. In addition, the Division administers a Laboratory Animal Medicine residency program for veterinarians and trains approximately five veterinary student in terns each year. Dr. Rudolf P. Bohm, Jr., Associate Director for Veterinary Resources at the TNPRC and Associate Professor of Clinical Medicine at Tulane Medical School, is Chairman of the Division.

The functions of the Division can be divided into three main areas: 1) Research, 2) Research Resources and 3) Education and Training Opportunities.

Research

The research component of the Division encompasses collaborative and independent work in a number of areas. Research focuses on reproductive biology, behavioral biology, diagnostics and treatment of spontaneous disease in NHP, infectious disease, and development of new animal models and research techniques in nonhuman primates (NHP).

Reproductive Biology

The reproductive biology research program is administered through the Unit of Reproductive Biology. Principle directions of research in reproductive biology include using state-of-the-art methods to expand a valuable rhesus monkey model of genetic disease, reproductive senility in women, and exploration of assisted reproductive technologies that will benefit individuals with impaired fertility.

Behavioral Biology

Tailoring behavioral management to rearing and research

Research must keep pace with elaborations to environmental enhancement programs in order to guide program evolution toward optimal benefit for the individual primate. This project provides direct benefit to the well-being and management of the Tulane National Primate Research Center colony, as well as to the Center's ability to make science-based management decisions. Rhesus macaques are being studied due to

their widespread use in biomedicine and the need for enhanced management of monkeys with varied rearing and research backgrounds. Project aims focus on social grouping and human interaction as enrichment. This project will provide valuable information for decisions relating to our ever-expanding and evolving environmental enhancement program.

Clinical Research

Clinically based research is performed in the Division of Veterinary Medicine to investigate improved diagnostics and treatment modalities for naturally occurring disease in nonhuman primates. The information that is generated in these studies improves the quality of life of animals at this and other institutions. In addition, clinical research is performed to develop new animal models and to improve the usefulness of current animal models.

Several studies are ongoing and include: the use of alternative anthelmintics to treat common intestinal parasites of nonhuman primates, the development of minimally invasive (endoscopic) surgical techniques for research and clinical use, the development of strategies to prevent and treat tetanus, and the assessment of the efficacy of new analgesic therapies.

Infectious Disease Research

AIDS comprises the largest component of the infectious disease research program within the Division of Veterinary Medicine. Research projects involving nonhuman primate models of AIDS and other infectious disease models are performed in collaboration with principle investigators from other Divisions and outside institutions. Current directions of research in infectious disease involve HIV breast milk transmission, HIV pathogenesis, HIV infection and alcohol abuse, West Nile virus disease pathogenesis and vaccine development, and SARS vaccine development.

Research Resources

The Division has a significant service commitment to the Center, and these service functions can be divided into four major areas: 1) Animal colony management and care, 2) Clinical imaging, 3) Surgical support, and 4) Assisted reproductive technologies.

Animal Colony Management and Care

Animal colony management and care is provided through the cooperation of several different units within the Division of Veterinary Medicine including Clinical and Research Medicine, Environmental Enrichment, Research Resources, and Animal Resources. The TNPRC animal colonies include the research colony and the breeding colonies. The breeding colonies are further separated into the conventional breeding colony and the specific pathogen free (SPF) colonies. The specific pathogen free colonies are comprised of animals that are free of targeted viruses that have the potential to affect health or could confound infectious disease research.

Nine species of nonhuman primates are represented at the TNPRC for a total population of approximately 5000 nonhuman primates. The represented African species are sooty mangabey (*Cercocebus torquatus atys*), white crowned mangabey (*Cercocebus torquatus lunulatus*), African green monkey (*Chlorcebus aethiops*), patas monkey (*Erythrocebus patas*), and baboon (*Papio* spp). Macaque species consist of rhesus monkey (*Macaca mulatta*), pigtail macaque (*Macaca nemestrina*), and cynomolgus macaque (*Macaca fascicularis*). The TNPRC has both Chinese origin and Indian origin *M. mulatta*. These regional variants have been maintained as separate populations in the breeding colony. The Center also houses a small number of squirrel monkeys (*Saimiri sciureus*) maintained for malaria research.

Animal Resources

The Unit of Animal Resources is a service unit that provides routine husbandry care for the animal colonies at the Center. The Unit also has responsibility to provide support to the Units of Clinical and Research Medicine, Environmental Enrichment and Research Resources, and to core and affiliate scientists. The Unit assists the Center in complying with relevant regulatory requirements including, but not limited to, those of the USDA, Public Health Service (PHS), and the U.S. Fish and Wildlife Service.

Routine husbandry practices include the reporting of any abnormal clinical sign or activity by animals to the appropriate veterinary medical staff and faculty. Animal Care Technicians provide support during diagnostic and therapeutic procedures and the administration of the preventive medicine program. The Unit closely coordinates its activities with research personnel to provide assistance, equipment and support for their work. The Unit of Animal Resources provides after-hours care, which includes administration of treatments, collection of biologic samples for research activities and observation of animals. The TNPRC facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

The Unit's staff consists of a Vivarium Manager, a Breeding Colony Manager, Resource Manager, two Quality Assurance Specialists, Animal Care Supervisors, and Animal Care Technicians.

Preventive Medicine

All nonhuman primates are acquired from established USDA registered nonhuman primate importers. A veterinarian examines all animals entering the TNPRC after arrival in the quarantine facility. During the quarantine period, a minimum of three TB tests are performed in addition to Primagam testing, fecal examination for parasites, rectal swab bacterial culture, serum chemistry, complete blood count, thin and thick blood smears for parasites, and testing for type D simian retrovirus. At each sample collection time point, a veterinarian performs a body weight measurement and physical examination. Prior to release from quarantine, all animals must have a normal thoracic radiograph, negative TB skin test and negative Primagam test.

Surveillance, Diagnosis, Treatment and Control of Animal Diseases

Animals are housed and separated based on species and infections they have encountered. Prior to assignment to research protocols, all nonhuman primates have a physical examination performed by the veterinarian assigned to the research project to determine fitness for the particular study. A specific veterinarian is assigned to each research project at the time of IACUC review. This procedure allows continuity in the provision of care to individual animals.

Breeding colony animals are housed in outdoor social groups. A minimum of twice yearly, all animals in a social group receive tuberculin testing, reproductive evaluations, and examination for pregnancy. Body weights, physiological samples, and demographic data are collected, and genealogical records are updated. Data collection and entry into the computerized animal records database allows the careful analysis of breeding colony production and clinical data.

Environmental Enrichment

The Unit of Environmental Enrichment is dedicated to improving nonhuman primate well-being through collaboration with the units of Clinical and Research Medicine and Animal Resources. The Tulane Environmental Enhancement Plan involves a number of strategies that are implemented according to animal needs and research requirements. The Plan is dynamic, permitting modification of techniques in accordance with in-house assessments and the scientific literature. New items are added to the program through an approval system including veterinary staff, animal care supervisory staff, and the Enrichment Coordinator. Conspecific social contact is the most critical element of the enrichment program, in recognition of the social nature of nonhuman primates. The socialization program places nonhuman primates into social groupings when compatible with research protocols, and dedicated staff monitors social introductions, ongoing compatibility, and social group dynamics. Other elements of the enrichment program include nonhuman primate/human positive interaction, feeding enrichment, structural enhancements, manipulable objects, and devices permitting foraging, grooming, problem-solving, and sensory enrichment. Several enrichment techniques are utilized concurrently with each individual nonhuman primate, scaled to the number and intensity of other feasible elements. Daily enrichment is implemented by Animal Care Technicians and Environmental Enrichment personnel.

Animal Resource Allocation

The Tulane Resource Allocation Committee (TRAC) was created to evaluate all proposed research projects that request utilization of the resources of the Center. The Committee, composed of ten members, includes research scientists, veterinarians, program coordinators and the animal colony epidemiologist. Several members represent facilities and programs from outside the Center. Requests are reviewed after both IACUC approval and funding is in place. Once TRAC approval is in place, the Division of Veterinary Medicine assigns the animal or space resource as it becomes available. Since the inception of the TRAC in 2001, animal allocations to affiliate (outside) investigators has been approximately 60% of the total, with the remainder allocated to core (inside) investigators.

Serum Bank

The TNPRC Rhesus Monkey Serum Bank is maintained by the Division of Veterinary Medicine and stores serum and plasma samples collected during routine veterinary care procedures for the breeding colony. The serum bank also includes samples collected during routine monitoring of viral status for the SPF colony. The purpose of the serum bank is to provide samples for retrospective analysis of the colony and for investigator use, if required, to minimize the need to access animals from the colony for serum samples. The samples have been catalogued and entered into the Center's database.

Breeding Colony Management

The Breeding Colonies of the TNPRC provide nonhuman primates to core investigators and affiliate investigators for research. The breeding colonies make up the largest population of nonhuman primates at the TNPRC. All animals in the colony are tracked via a centralized, computerized animal records system. With

exception of the animals housed for treatment of illness, the animals assigned to the breeding colony are housed in outdoor enclosures in social groups. Social groups are housed in large fenced corrals and field cages that allow for the establishment of a normal social dynamics similar to that found in feral troops. The breeding colony management program is designed and administered by veterinarians, the breeding colony manager, environmental enrichment coordinator, and the breeding colony epidemiologist through the Breeding Colony Management Committee. In addition, the Tulane Resource Allocation Committee (TRAC) facilitates breeding colony management by determining appropriate allocation of animals for assignment to research protocols based on statistical analysis of colony demographics.

The demand for animals from the SPF and conventional colonies has increased dramatically over the past five years. Requests from investigators reflect the need for more thoroughly characterized nonhuman primates, with regard to viral status and genetic background. Our long-range goal is to expand the breeding colonies so that all animals are SPF.

Clinical Imaging

Radiology

Radiology support is provided with a Continental 150KV, 300 mA fluoroscopy unit with image intensification. A direct digital radiography system is utilized to capture images to a picture archiving system (PACS) server. Approximately 700 radiographs are taken each year.

Ultrasound

Ultrasonographic examinations and procedures are performed using one of three Toshiba or a portable GE Logicbook ultrasound machine. Color doppler capability is present on all of the machines.

MRI

MRI is performed on the TNPRC campus on a contract basis utilizing a private imaging company.

Surgical Support

Surgery is performed in either of two fully-equipped operating rooms. Surgical facilities are under the direct supervision of a veterinarian, who is assisted by the surgery supervisor and two surgery technicians. Procedures performed are those approved by the IACUC and/or administered for the medical management of non-research animals.

Assisted Reproductive Technologies (ART) Core

The ART Core is administered through the Unit of Reproductive Biology in the Division of Veterinary Medicine. The program is focused on several areas of embryo production and embryo manipulation. The significance of ART in rhesus monkeys for biomedical research is growing with rapid advances in production, preservation and manipulation of embryos. These advances may ultimately facilitate genetic modification of embryos, the identification of embryos with specific genotypes, or the production of identical twins by embryo splitting.

Timed Breeding Program

This program is used to provide timed bred rhesus monkeys for investigator use in approved research protocols.

Education and Training Opportunities

The Unit of Clinical and Research Medicine within the Division provides for the administration and oversight of the Laboratory Animal Medicine Preceptorship Program, Laboratory Animal Medicine Residency Program and the Division of Veterinary Medicine Training Committee.

Laboratory Animal Medicine Preceptorship Program

Veterinary students enrolled in the professional curriculum and postgraduate veterinarians participate in the program. Our training program exposes veterinary students and graduate veterinarians to all aspects of the research environment, including regulatory issues, research support, colony health surveillance, clinical medicine, and surgery. In addition, the Division of Comparative Pathology at TNPRC provides instruction in pathological findings of spontaneous and experimental disease in nonhuman primates to these students. On average, five students per year are mentored through this program.

Laboratory Animal Medicine Residency Program

The integration of the Division into the American College of Laboratory Animal Medicine (ACLAM) accredited Laboratory Animal Medicine Residency program at the LSU School of Veterinary Medicine (SVM) provides for a full time resident position at the TNPRC that focuses on nonhuman primate medicine and surgery. The in-house residency program runs concurrently with the Laboratory Animal Medicine Preceptorship Program (described above), which provides opportunities for students outside of the Tulane/LSU system. Only a few ACLAM-accredited residency programs exist that offer a similar exposure to nonhuman primate medicine. The duration of the program is two years and provides eligibility for board certification by ACLAM. Residents spend nine months of each year at the TNPRC, with the balance spent at the LSU SVM for didactic course work including pathology of laboratory animals. Residents are required to act as the principle investigator of a research project, which is mentored by senior faculty at the TNPRC. Attendance at seminar series, grand rounds, histopathology rounds, journal club, IACUC, Breeding Colony Management and TRAC meetings are additional mandatory components of the training.

Training Committee

The Training Committee of the Division of Veterinary Medicine provides training to employees of the Division. The training committee membership consists of veterinarians, management personnel, technicians, quality assurance personnel and the TNPRC Occupational Health and Safety Nurse. A series of training modules using PowerPoint presentations for topics such as occupational health and safety, use of personal protective equipment, disease control measures, animal observations, environmental enrichment, review of routine husbandry practices, and anesthesia in nonhuman primates were created and are presented at Division meetings. The training program for new staff requires a rotation for each new employee through the various components of the Division of Veterinary Medicine prior to final assignment.

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