FACULTY RESEARCH INTERESTS
of the
DEPARTMENT of MICROBIOLOGY
and IMMUNOLOGY
2015

Figure: Tat Protein of Neurovirulent HIV-1 Attenuates Neuronal Dendrites

Image provided by: Dr. Vinayaka Prasad
Message from the Chairman

It is a great pleasure for me to welcome you to the Sue Golding Graduate Division (SGGD) of the Albert Einstein College of Medicine. Our laboratories in the Department of Microbiology and Immunology cover a wide range of topics spanning the fields of virology, bacteriology, host immune responses to infection and cancer, inflammation and autoimmune disease. The department warmly and enthusiastically welcomes PhD candidates who are interested in pursuing research projects and potentially developing careers in these areas. I welcome all new students in the SGGD to contact me or any of the department's faculty directly to explore the terrific range of opportunities available to you in the Department of Microbiology and Immunology.

My best wishes for success in your new adventure!

Professor & Chairman
Department of
Microbiology and Immunology
## MICROBIOLOGY AND IMMUNOLOGY

### FACULTY

#### 2015 – 2016

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## GRADUATE STUDENTS
### 2015 – 2016

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Dr. Berman’s laboratory examines the mechanisms that mediate HIV entry into the CNS and how viral and inflammatory mediators damage neurons and other CNS cells. More than 40 million people worldwide are HIV infected. As a result of antiretroviral therapies, HIV infected people are living longer. HIV enters the CNS early after infection and despite therapy, persists within the CNS. Prevalence of NeuroAIDS and its associated cognitive impairment is increasing. An understanding of mechanisms that mediate these effects are critical to the development of therapeutic strategies.

HIV infection of the CNS can have devastating consequences, often resulting in cognitive impairment and severe neurological complications. The basis of this impairment is poorly understood. Although its development is associated with early viral infiltration of the CNS, the number of activated monocytes/macrophages within the CNS appears to be a better indicator of neurologic compromise than viral load, suggesting that leukocyte infiltration and cognitive impairment are tightly correlated. How infected monocytes cross the blood brain barrier (BBB) and infiltrate the CNS is not well understood. This process is critical to the development of NeuroAIDS as it brings leukocytes into the brain where they activate and infect microglia, and affect damage to the BBB and other CNS cells. The mechanisms of HIV-infected monocyte transmigration across the BBB have only been minimally characterized. We are characterizing several of the steps in this transmigration process using a tissue culture model of the human BBB. We analyze the mechanisms that mediate attachment and diapedesis of HIV-infected monocytes across the BBB to identify markers that contribute to brain infection and BBB disruption, such as adhesion molecules, tight junction and adherens proteins, chemokines and their receptors. The lab has a major translational component, examining sera and CSF from HIV infected individuals for predictors of cognitive impairment, as well as patient cells for unique markers of this impairment and for their ability to transmigrate across the blood brain barrier. We examine tissue from HIV-infected individuals for altered proteins. The overall goal is to identify targets for therapeutic intervention to limit the entry of HIV into the CNS.

Many HIV-infected people who abuse drugs have more extensive CNS damage associated with significant cognitive impairment. As many drugs of abuse cause an increase in extracellular dopamine, we examine the effects of dopamine on HIV infection of macrophages. We demonstrated that dopamine increases HIV infection of human macrophages and are addressing the mechanisms by which dopamine causes this increase as well as alterations in macrophage function. We also study the impact of buprenorphine and methadone, therapies for Opiate abuse, in the context of NeuroAIDS.

**PUBLICATIONS**


astrocytes disrupts blood brain barrier integrity by a gap junction dependent mechanism. The Journal of Neuroscience. 31(26):9456-65.


DR. ROBERT BURK

Human Papillomavirus (HPV) Translational Science: Molecular Epidemiology, Pathogenesis and Evolution.

The main focus of the Burk laboratory is to understanding how evolution of papillomaviruses has resulted in the emergence of HPV types that are highly pathogenic and cause multiple cancers in humans (e.g., cervix, head & neck, skin). In addition, the lab is also exploring the role of epigenetic changes in the viral genome and its relationship to precancer and cancer development. The role of the cervical microbiome is being evaluated as another exposure in the same studies. These investigations extend from clinical studies where we obtain exfoliated cervix cellular material (Pap cells) and evaluate the HPV genome, CpG methylation and the composition of the microbiome to cell based biochemistry studies of viral proteins and molecular evolution of viral sequences.

The papillomaviruses are 8.0 kb double stranded DNA viruses readily amenable to amplification and sequencing, making this system ideal as a model for DNA virus evolution and identification of pathogenic genetic signatures. Over 200 HPV types exist and further characterization of HPVs infecting the population (i.e., from our large sample repository) have allowed us to explore the virus as a species, characterization of the frequency and heterogeneity of HPV types and variants in the population, and the role of viral evolution in pathogenicity. The lab uses phylogenetic methods and other analytic strategies to test hypotheses about the relationship and characteristics of HPV genomes and disease. Exploration of natural selection of papillomaviruses has led us to the conclusion that the viruses are evolving through complex means yet to be discovered. Our major collaboration with investigators at the National Cancer Institute, NIH has provided an ideal translational team of world-class epidemiologists, biostatisticians and clinical investigators. In combination with evolutionary biologists at the American Museum of Natural History, our integrative group provides a unique prospective for intellectual growth of students that want to “think outside the box”. More recently, we have investigated epigenetic changes to the HPV genome and have demonstrated very significant results on the association of these changes with neoplastic progression. The identification of HPV in specific biological niches has challenged us to explore the microbiome through barcoding and parallel sequencing using Next-Gen methods. We have recently developed the methodologies and computer software to test hypothesis on the influence of the microbiome on cervix cancer development in HPV positive women.

Other research areas include a human genetic project to identify the gene(s) for hyperhidrosis (excessive sweating). This is a fascinating disorder that is strongly associated with a family history of excessive sweating. There seems to be at least two phenotypes, excessive sweating from the palms and soles, and excessive sweating from the underarms, body, face and groin areas. The analyses of families with this disorder suggest genetic and/or allelic heterogeneity. To date, we have collected over 1500 DNA samples from affected individuals and families. We are in the process of exome-sequencing the coding regions using Next-Gen technologies to identify candidate mutations associated with this disorder.

Lastly, as part of our goal to understand genes and cancer, we have for many years studied the von Hippel-Lindau (VHL) gene that is a driver of kidney cancer. We investigate the function of the VHL protein, in part, an oxygen sensor. Our recent observations indicate that intact VHL is required for primary cilia formation and function in renal cells. We have localized VHL and other proteins known to interact to cilium. Further studies will investigate the function of VHL in the cilium.
PUBLICATIONS

Human Papillomavirus


**Other studies (VHL, hyperhidrosis and prostate cancer)**


Our lab studies how *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (TB), interacts with the host; on-going studies include:

**Tuberculous latency and reactivation:** Gene profiling studies have identified a set of Mtb genes (the dormancy regulon) that are regulated by exposure to low oxygen tension and nitric oxide, two latency-promoting environmental signals. Deletion of a dormancy regulon gene, *rv2623* (a member of the USP [universal stress protein] family), results in hypervirulence in infected hosts. *Rv2623* also regulates Mtb growth in vitro. The growth-regulating attribute has been linked to the ATP-binding capacity of *Rv2623*. Understanding how *Rv2623* regulates the growth of Mtb and how members of the dormancy regulon promote latency is a focus of our laboratory.

We are collaborating with JoAnn Tufariello to study the role of the five Mtb Rpf proteins, putative peptidoglycan hydrolyases, in regulating tuberculous latency and reactivation. Mutagenesis studies showed that Mtb doubly deficient for RpfA and RpfB displays defects in persistence and reactivation, both in vivo and in vitro models of tuberculous latency and disease recrudescence. Studies have been initiated to characterize how Mtb Rpf’s regulate mycobacterial growth.

**The mechanisms that regulate the granulomatous reaction:** Our Studies using the B cell-deficient µMT mouse model and specific Fcγ receptor knockout mice have shown that humor immune response regulates the tuberculous granulomatous reaction and is required for optimal control of Mtb. There is also evidence that B cells can regulate T cell response in infected hosts. We are conducting experiments aimed at characterizing the roles of B cells in regulating the host granulomatous response to Mtb.

TNF is essential in controlling TB. However, TNF also contributes to the development of immunopathology. TNF neutralization reactivates TB in both mice and humans with persistent infection. We are using this TNF neutralization reactivation model to study the role of TNF in regulating the tuberculous granulomatous reaction. We are also generating cell-specific conditional TNF knockout mouse strains that will be used to decipher the relative contribution of cell-specific TNF in the protective immunity and immunopathogenesis of this cytokine in the host.

**TB vaccines:** We will apply the knowledge gained from the above-listed investigation to rationally design safe and effective TB vaccines in collaboration with the Jacobs and Porcelli labs. For example, understanding how to manipulate the humoral response to the advantage of the host may lead to the development of vaccines with increased efficacy. Knowing how Mtb regulates host production of TNF may lead to the design of Mtb-derived vaccines with enhanced immunogenicity.

**PUBLICATIONS**


The overarching goals of my lab are twofold. First, we seek to make a ‘molecular movie’ of the process by which the highly pathogenic Ebola and Marburg filoviruses gain entry into the cytoplasm of host cells, where all the ‘goodies’ for viral multiplication are located. Second, we seek to exploit this knowledge to develop new anti-filovirus therapeutics.

The filovirus entry mechanism is unusually complex (i.e., interesting!). It consists of multiple steps in which the virus interacts with and co-opts distinct host cell molecules, and is itself structurally transformed as a result. Over the last few years, we and others have found that filovirus entry is profoundly dependent upon the cellular endocytic pathway. First as a postdoc, and then as a faculty member at Einstein, I have helped to identify endosomal host factors and pathways that are critical for filovirus entry and potential targets for antiviral therapy. In 2005, I showed that the endosomal cysteine proteases cathepsin B and cathepsin L are required for viral entry and act by cleaving the viral glycoprotein. Very recently, grad students Tony Wong and Emily Miller in my lab have made a remarkable discovery. They have shown that the Niemann-Pick C1 protein, a highly-studied cholesterol transporter in lysosomes, is indispensable for filovirus entry and acts as a receptor that interacts with the filovirus glycoprotein. Satisfyingly, Emily has found that GP must first be cleaved by cathepsins B and L before it can interact with NPC1 (see model). NPC1 exemplifies a type of viral receptor that is new to virology – it interacts with its viral ligand only within the endocytic pathway and not at the cell surface. The identification of NPC1 as an intracellular filovirus receptor promises to revolutionize our understanding of the filovirus infection cycle, in vivo viral pathogenesis, and the ecology and natural history of filovirus infections. Not least, NPC1 provides a new target for the development of anti-filovirus therapeutics.

**A model for filovirus entry.** Virus particles adhere to the plasma membrane by binding to one of several cell attachment factors. They are then trafficked to early/late endosomal compartments (EE/LE) where host cysteine proteases, including cathepsin B, cleave GP to remove the mucin and glycan cap domains and expose the NPC1-binding site. This cleaved GP species, which resembles in vitro-cleaved GPCL, can then bind to NPC1 on the limiting membrane of a late endosomal (LE) or lysosomal (Lys) compartment. GP-NPC1 interaction acts alone or in concert with unknown endo/lysosomal host factor(s) to trigger GP conformational changes that bring about viral membrane fusion and cytoplasmic release of the viral nucleocapsid payload.

Current questions include:
1. Why is the Niemann-Pick C1 (NPC1) protein required for filovirus entry?
Using biochemical, genetic, cell biological, and structural approaches, we are defining the virus-NPC1 interaction, determining its location within the host cell, and trying to understand what binding to NPC1 accomplishes for the virus. Our working hypothesis is that NPC1 induces structural changes in the viral glycoprotein that drive fusion of viral and endosomal lipid bilayers and bring about viral escape into the host cytoplasm.

2. What role does NPC1 play in determining viral host range and transmissibility?
Filoviruses persist in bat reservoirs and are only occasionally transmitted to human and primate hosts with devastating consequences for the latter. We are asking if the initial encounter between virus and NPC1 can govern, or predict, filovirus host range and inter-species transmission, as is observed for other viral pathogens and their receptors (e.g., HIV, SARS, flu).

3. What role does NPC1 play in filovirus in vivo pathogenesis?
In collaboration with the Walkley Lab at Einstein, and John Dye's group at USAMRIID, we are using constitutive and inducible NPC1 knockout mice to ask if NPC1 is required for filovirus multiplication in vivo and viral hemorrhagic fever.

4. Can antivirals targeting NPC1 be developed?
Chemical screens for filovirus entry inhibitors that I carried out as a postdoc led to the discovery of a small molecule that binds to NPC1 and inhibits filovirus infection. We are working with an industry partner to identify other novel small molecules that disrupt the virus-NPC1 interaction and to develop a lead compound for in vivo trials.

5. How do endosomal cysteine proteases mediate filovirus entry?
We have found that specific endosomal cysteine proteases (cathepsins B and L) are essential host factors for filovirus entry and new targets for development of antiviral drugs. We have shown that glycoprotein cleavage by these enzymes during entry is needed not only to expose a binding site for the viral receptor NPC1, but also for the induction of conformational changes that bring about viral membrane fusion. We are using a combination of approaches to test our working hypothesis, which is that filoviruses employ proteolysis in combination with receptor binding to trigger viral membrane fusion.

6. What additional host factors and pathways are required for filovirus entry?
The same genetic screen that uncovered NPC1 also identified other host pathways that are critical for filovirus entry. These include multiprotein complexes that are involved in the biogenesis and trafficking of specific endosomal compartments. We are using mutant cell lines deficient in these and other cellular proteins, together with confocal and electron microscopy, to directly examine the endocytic route of filovirus entry, and to identify compartments in which the different entry steps occur. We are also carrying out new genetic and chemical screens to expand the catalog of filovirus entry factors.

7. What is the cascade of structural transformations in the viral glycoprotein that drives filovirus entry?
In collaboration with the Jon Lai lab at Einstein, we are using state-of-the-art synthetic antibody repertoires and phage display technology to develop monoclonal antibodies that bind to different conformational states of the filovirus glycoprotein. Also in collaboration with the Lai Lab, we have engineered novel intracellularly-targeted peptides that bind to structural intermediates in the membrane fusion reaction. We hope these leverage these reagents to illuminate new frames in our molecular movie of viral entry. In addition, both antibodies and peptides represent possible strategies for therapeutic intervention.

**PUBLICATIONS**

*Corresponding author

**NPC1 and other novel host factors for Ebola virus entry:**


**Endosomal cysteine proteases in Ebola virus entry:**


**Cell biology of Ebola virus entry:**

Structure and function of the Ebola virus glycoprotein:


Novel antiviral strategies:


Review articles:

Molecular mechanisms of neurodegeneration in animal models

Previous work from my laboratory at the NIH, uncovered a role in programmed cell death for the APP intracellular domain (AID/AICD) that is released after APP-derived substrates are processed by the g-secretase in the transmembrane region. We are studying the functional roles of AID both in vivo, using APP knock in and AID transgenic mice, and in vitro.

The lab is also interested in studying regulator of APP processing. We have identified BRI2 as an APP ligand that prevents APP processing. BRI2 is also mutated in genetic forms of dementias, namely Danish and British dementias (FDD and FBD). We have generated BRI2 transgenic and knock out animals, as well as knock in animals expressing pathogenic FDD and FBD BRI2 mutants. These animals are used to study the mechanisms by which BRI2 regulates APP processing. Our data so far suggest that the dominant hypothesis for the pathogenesis of neurodegenerative diseases, the “Amyloid Cascade Hypothesis” is not correct, and suggest an alternative mechanism of pathogenesis for human dementias.

PUBLICATIONS


Dr. Ekaterina Dadachova

Dadachova’s laboratory exists at the Albert Einstein College of Medicine since the end of 2000. We are interested in targeted radionuclide therapy of cancer and infectious diseases and radiation protection materials. Radionuclides emit particles capable of destroying the cells. Monoclonal antibodies are perfect “vehicles” for targeted delivery of radionuclides to the cancerous or microbial cells. Such modality is called radioimmunotherapy (RIT). RIT is currently routinely used in the clinic for treatment of some cancers and dozens of clinical trials are also being conducted worldwide.

1. Novel treatments for metastatic melanoma. We are targeting melanin in tumors with adiolabeled melanin-binding antibody as a basis of therapy for currently incurable metastatic melanoma. Since mid-2001 when we started this work - the RIT of melanoma with melanin-binding 6D2 mAb has made into the clinic and Phase I/II clinical trial has been completed with the encouraging results and reported at the 2011 meeting of the American Society for Clinical Oncology and published in 2013. In the lab we are currently evaluating interaction of RIT with melanoma stem cells in order to design more effective treatment protocols for the patients.

2. Radioimmunotherapy of HIV and fungal infections. RIT for infection is theoretically useful for any microbe susceptible to radiation killing including bacteria, fungi, viruses and parasites. One could anticipate that targeting microbes will be easier than targeting neoplastic cells given the enormous antigenic differences between host and microbe. Currently we are investigating the possibility of treating invasive fungal infections with radiolabeled antibodies to universally shared fungal antigens to avoid the lengthy diagnosis of the disease in immunocompromised patients. Bill and Melinda Gates Foundation funding enabled us to study the interaction of anti-retroviral therapy (ART) with RIT targeting HIV-infected human cells as a prelude for the clinical trial of RIT of HIV in patients.

3. Radioimmunotherapy of cancers of viral origin. We have pioneered the use RIT for targeting viral antigens in cancers of viral origin such as HPV-related cervical or head and neck cancers and Hepatitis B-related hepatocellular carcinoma. The fundamental novelty of this approach to treat cancer as an infectious disease is that the viral antigens are very different from “self” human antigens thus providing unmatched specificity of the treatment and simultaneously minimizing toxicity to normal tissues. We are collaborating on this project with Dr. R. Phaeton, a physician-scientist working in the field of gynecological oncology at the Hershey Medical Center, Penn State University.

4. Melanin pigment as an energy transducer and radioprotector. We are investigating melanin’s energy transduction properties with one of the purposes of this work being to create novel materials for internal and external radiation protection of patients undergoing radiation therapy of cancer as well as in case of radiological accidents like "dirty bomb". This project also involves expanding our observations on the fundamentally new role which elain in can play in melanized microorganisms by converting the energy of ionizing radiation into the chemical energy usable in a microbial cell life cycle - the process which we called "radiosynthesis" by analogy with photosynthesis in green plants. Our first paper on this subject appeared in PLOS ONE 2007 and caused enormous interest with more than 100,000 people eading it just on the journal’s website

http://www.plosone.org/article/metrics/info%3Adoi%2F10.1371%2Fjournal.pone.0000457; jsessionid=6223B838B84A9DDE451AFB18834499BB.ambra02
Our current research in this field is funded by the Defense Threat Reduction Agency.

5. Radioactive Listeria for treatment of pancreatic cancer. In collaboration with Dr. Claudia Gravekamp from the M&I we created a radioactive Listeria monocytogenes bacteria which can carry radionuclides into the tumors and destroy them by combined effect of reactive oxygen pecies and radiation. Such therapy is very promising for highly aggressive cancers with poor prognosis such as pancreatic cancer.

PUBLICATIONS


Our primary research interest is in pathogenesis in the *Plasmodium falciparum*. Patients infected with this parasite can be completely asymptomatic or develop severe disease resulting in death. The goal of our research has been to define the molecular mechanisms that underlie this variation in disease outcomes in *P. falciparum*. Toward this goal, we have developed a new pathogenesis model through the analysis of *in vivo* parasite biology and associated host factors using a whole genome approach. We have identified novel parasite biology when it resides in the human host; this biology has not been reported under *in vitro* cultivation and may play a role in enhanced virulence and/or transmission capacity. Using longitudinal studies we have characterized individual responses to malaria during a severe and subsequent mild infections to understand how patients immune responses may change and lessen immunopathology. The long term goal is to identify parasite and host processes involved in disease to serve as targets for vaccine or chemotherapeutic development. We carry out field based translational studies in cohorts infected with malaria in Africa and these inform our experimental work using basic molecular biology approaches in the laboratory.

I. Recapitulation of in vivo state: To further understand the implications of these novel *in vivo* states we will study the parasite under *in vitro* conditions that mimic host blood stream conditions.

II. Define host response to infection: We are also studying host response to infection using whole genome approaches to identify host factors that associate with severe disease outcomes.

III. Comprehensive analysis of parasite produced small molecules: We identified parasite specific small molecules to study its biology using a complementary approach and potentially these molecules could serve as biomarkers of infection.

**PUBLICATIONS**


Type 1 diabetes is an organ-specific autoimmune disease characterized by T cell-mediated destruction of the insulin-producing beta cells of the pancreatic islets. While insulin therapy allows for continuation of life, it neither cures the disease nor prevents its devastating complications. Studies utilizing the nonobese diabetic (NOD) mouse model of the disease have shown that T cells, recognizing autoantigenic peptides bound to major histocompatibility complex (MHC) molecules, are absolutely required for disease development. T cells specific for beta cell antigens can also be detected in the peripheral blood of type 1 diabetes patients. Our laboratory utilizes an extensive collection of mouse models to investigate the antigenic specificities, pathogenicity, and immunobiology of T cells in type 1 diabetes. These models are also being used to develop and optimize therapeutic strategies, and new, increasingly “humanized” mouse models are continually in development in our group. The goals of our work are to better understand the underlying immunopathogenesis of type 1 diabetes and to develop improved tools to monitor and manipulate pathogenic beta cell-specific T cells.

**PUBLICATIONS**


DR. MARTA FELDMESSER

We are developing antibody-based reagents for prevention and diagnosis of disease due to Aspergillus. Invasive aspergillosis occurs mainly in severely immunocompromised hosts and our ability to diagnose or treat this disease with antifungal drugs is limited. Therefore, strategies that enhance the host immune response are an attractive area for development. The laboratory is developing monoclonal antibodies (MAb) that block or delay germination, the transition from the spore form to hyphal growth that is required for A. fumigatus to invade host tissue. Currently, we are focused on one MAb that we made (MAb 318) that inhibits germination in vitro, alters alveolar macrophage-conidia interactions and prolongs survival in experimental murine pulmonary infection. MAb 318 binds to three A. fumigatus proteins. We are currently distinguishing which interaction inhibits germination. We are also examining mechanisms by which MAb 318 enhances macrophage function and prolongs survival. Long term goals include determining the suitability of MAb that bind to this target for passive prophylaxis and of the target itself as a vaccine to prevent disease. We also want to understand the role of this protein during germination and identify additional MAbs that prevent germination, as complete inhibition may require binding to more than one target.

A second area of investigation is development of better antibody-based diagnostic tests for invasive aspergillosis. Currently, the most commonly assayed biomarker is serum galactomannan, a cell wall carbohydrate released during invasive disease. The Biorad Platelia™ Aspergillus Assay, approved by the U.S. Food and Drug Administration for this use, is limited in both sensitivity and specificity. We are applying antibody engineering techniques to this problem. We made five MAbs that bind galactomannan, allowing its detection in serum and bronchoalveolar lavage fluid from infected mice by capture ELISA. The variable region sequences of these MAbs are very close (≥94 % identical) to those of germline genes. Our highest affinity MAb has the largest number of amino acid substitutions and these are located in a complementarity determining region. We are currently employing antibody engineering techniques to increase the affinity of this MAb. We selected a strategy for affinity enhancement that uses a series of methods for mutation, selection and display that, when used in combination, have allowed 10,000 fold augmentation. Higher affinity reagents will be tested for their ability to diagnose invasive aspergillosis in animal models in comparison to the Platelia™ Aspergillus Assay. Those with improved sensitivity and specificity will be assessed for their ability to improve diagnosis of human disease.

We also are developing MAbs to inhibit A. fumigatus and A. parasiticus aflatoxins, common contaminants of stored grains that are potent hepatic carcinogens and acutely toxic. No protective MAbs against aflatoxin exposure have been identified. We made a novel aflatoxin-keyhole limpet hemocyanin conjugate that is potently immunogenic in rats. We are currently making MAbs to aflatoxin B1. MAbs will be tested for their ability to prevent acute toxicity and DNA damage in vitro and in vivo. In the long term, protective MAbs will be assessed for capacity to prevent disease in humans.

PUBLICATIONS


The goals of my laboratory are to understand the regulation of plasma cell differentiation, migration, survival and function. Plasma cells are terminally-differentiated B cells that secrete high-affinity antibodies constitutively, following immunization and exposure to a pathogen. The quality, magnitude and longevity of the antibody response are dependent upon the differentiation and survival of these cells, which involves many signaling factors and auxiliary cell types. We have used intravital two-photon imaging to study plasma cell differentiation and migration in the lymph node and have found that these cells exhibit a highly linear migration that is independent of $g_{\alpha i}$ chemotaxis. This migration is unique among lymphocytes and enables these cells to travel long distances crossing heterogeneous microenvironments to reach niches critical for their survival. In some cases, plasma cells may undergo malignant transformation during differentiation leading to neoplasms in humans such as multiple myeloma. Despite their critical role in immune function and disease, many fundamental questions remain regarding the physiology of plasma cells in vivo. We are using two-photon intravital imaging in combination with modern cellular and immunological tools to visualize and better understand the physiology of these cells under normal and pathological conditions. The current topics in the laboratory are focused on:

1. Plasma cell differentiation. What factors regulate selection and differentiation of germinal center B cells to plasma cell?
2. What factors control plasma cell migration to the bone marrow and subsequent long-lived survival and retention?
3. What factors control myeloma cell retention and migration in the bone marrow, which enables tumor progression?

**PUBLICATIONS**

(* corresponding author, # senior author)


**Fooksman DR**. Organizing MHC class II Presentation. Front. Immunol., 10 April 2014;


Our laboratory studies the cell biological processes and the molecular pathways involved in cardiac repair, remodeling and fibrosis. The adult mammalian heart has negligible regenerative capacity and heals through formation of a collagen-based scar. Repair of the infarcted heart is dependent on induction and timely suppression of inflammatory signals, and on recruitment of reparative cells (fibroblasts and vascular cells). Dysregulation of the inflammatory and fibrotic responses causes adverse remodeling of the heart and results in heart failure. Using cell-specific genetic manipulations, established mouse models of cardiac injury and remodeling, and cell biological assays (using isolated cardiomyocytes, fibroblasts and macrophages), we explore the molecular circuitry of myocardial repair and fibrosis. Ongoing studies address the following questions:

1. **What are the signals implicated in suppression and resolution of the post-infarction inflammatory reaction?**

Timely inhibition and spatial containment of inflammatory signaling are critical for cardiac repair. We study the role of macrophage-specific inhibitory signals in suppression and resolution of the post-infarction inflammatory reaction.

2. **Which the molecular signals are responsible for fibroblast activation and de-activation in infarcted and remodeling hearts?**

In the infarcted heart, fibroblasts critically regulate cardiac repair by transdifferentiating into myofibroblasts and by producing extracellular matrix proteins. However, excessive or dysregulated activation of fibroblasts results in extension of fibrosis and causes diastolic ventricular dysfunction. We study the molecular signals that activate and de-activate fibroblasts in cardiac repair, focusing primarily on the role and regulation of the TGF-beta cascade.

3. **How does the extracellular matrix modulate the phenotype of cells involved in repair and fibrosis?**

The extracellular matrix is not simply a structural scaffold, but actively participates in transduction of signaling responses. Specialized components of the matrix are induced following injury and modulate cytokine and growth factor-mediated responses, signaling through integrins or syndecan receptors. Our lab is particularly interested in the biology of these “matricellular proteins” in cardiac repair and remodeling.

4. **How does metabolic disease cause cardiac fibrosis?**

Diabetes and obesity are associated with profound alterations in cardiac function causing diastolic heart failure. Our lab studies the effects of metabolic dysregulation on cardiac fibroblasts and explores the mechanisms of fibrosis and capillary rarefaction in diabetic hearts.

5. **What is the fate and role of pericytes in the infarcted and remodeling heart?**

Pericytes are abundant in the mammalian heart and may regulate angiogenic and fibrogenic responses. Our lab will study the fate and role of pericytes in myocardial infarction and in diabetic cardiomyopathy using lineage tracing strategies and cell-specific loss-of-function approaches.
The ultimate goal of our research is to identify therapeutic targets for attenuation of adverse remodeling following cardiac injury, thus preventing the development of heart failure.

**PUBLICATIONS**

**On the role of inflammatory signaling in cardiac repair and remodeling:**


M Dobaczewski, Y Xia, M Bujak, C Gonzalez-Quesada, and NG Frangogiannis. CCR5 signaling suppresses inflammation and reduces adverse remodeling following myocardial infarction mediating recruitment of regulatory T cells. *Am J Pathol* 2010; 176: 2177-87.


AV Shinde and NG Frangogiannis. Mediators secreted by myeloid cells may protect and repair the infarcted myocardium *Circ Res* 2015; 117:10-12.


**On the biology of the fibroblast:**


A Saxena, M Bujak, O Frunza, M Dobaczewski, C Gonzalez-Quesada, B Lu, C Gerard and NG Frangogiannis. CXCR3-independent actions of the CXC chemokine CXCL10 in the infarcted myocardium and in isolated cardiac fibroblasts are mediated through proteoglycans. *Cardiovasc Res* 2014; 103: 217-227.


**On the biology of the matricellular proteins:**

Y Xia, M Dobaczewski, C Gonzalez-Quesada, W Chen, A Biernacka, N Li, D Lee and NG Frangogiannis. Endogenous Thrombospondin-1 protects the pressure-overloaded myocardium by modulating fibroblast phenotype and matrix metabolism. *Hypertension* 2011; 58: 902-911.


C Gonzalez-Quesada, M Cavalera, A Biernacka, P Kong, DW Lee, A Saxena, O Frunza, M Dobaczeewski, AV Shinde, and NG Frangogiannis. Thrombospondin-1 induction in the diabetic myocardium stabilizes the cardiac matrix while promoting vascular rarefaction through angiopoietin-2 upregulation. *Circulation Research* 2013; 113(12):1331-44.

**DR. BETTINA FRIES**

*Key Words:* C. neoformans, cryptococcosis, bioterrorism, toxins, virulence, cytokines, phenotype switching, replicative aging

The primary focus of my laboratory is the pathogenesis of chronic *cryptococcosis*. We demonstrated that *C. neoformans* manifests multiple phenotypes, which allows the yeast to evade the immune response. Research projects focus on the host response as well as the molecular mechanisms that allow the fungus to change. Using mouse models we established that phenotypic switching occurs *in vivo* and changes the outcome in chronic *Cryptococcus* infection. Microarray analysis of switch variants have determined that phenotypic switching to a hypervirulent switch variant involves epigenetic down regulation of several genes. We have generated knockout variants and this work confirms that a Knockout leads to hypervirulence. Now we will study the contribution of these genes to virulence and investigate molecular mechanisms that regulate this epigenetic silencing. Our work is of general importance because it studies genes that down regulate virulence rather than up-regulate virulence. We also examine an aspect of microbial pathogenesis that has never been studied before: the role of microbial aging in virulence. *C. neoformans* undergoes asymmetric cell divisions. *This process is referred to as generational or replicative aging.* As a consequence older, also referred to as senescent mother cells can be distinguished from younger daughter cells or virgin buds. The ensuing phenotypic changes in the aging mother are such, that they could potentially give older cells a biological advantage *in vivo*, thus promoting their selection. The ability to sample pathogen directly from the CSF, and the availability of serial isolates from CSF samples offers a unique opportunity to investigate this dynamic microevolution within a pathogen population, which replicates in a host with cryptococcal meningitis. In addition, this laboratory works on a bioterrorism related project. We study the relevance of staphylococcal toxins during natural *S. aureus* infection. We have generated monoclonal antibodies to staphylococcal enterotoxin B (SEB). These mAbs can protect SEB injected mice from SEB induced shock and death. In addition we have developed a capture ELISA that allows us to measure SEB toxin in body fluids. Studies now concentrate on making high affinity antibodies and chimeric antibodies that are optimized for toxin neutralization of SEB *in vivo*.

**PUBLICATIONS**


T. Bouklas , and **B. C. Fries**. *C. neoformans* constitutes an ideal model organism to unravel the contribution of cellular aging to the virulence of chronic infections. Curr Opin Microbiol. 2013 Apr 27

K Varshney, X. Wang, M. D Scharff, J. MacIntyre, R.S. Zollner, O. V Kovalenko, L. R. Martinez, F. R Byrne and **B. C Fries**. *Staphylococcal enterotoxin B specific monoclonal antibody 20B1 successfully treats diverse Staphylococcus aureus infections* JID August 6, 2013


A. Guerrero, N. Jain, X. Wang and B.C. Fries Cryptococcus neoformans variants generated by phenotypic switching differ in virulence through effects on macrophage activation Inf and Immun 2010 Mar;78(3):1049-57


N. Jain, L. Li, Y.P Hsueh, A. Guerrero, J. Heitman, D.L Goldman and B. C. Fries. Loss of Allergen1 (ALL1) confers a hypervirulent phenotype that resembles mucoid switch variants of Cryptococcus neoformans Infect and Immum 2009 Jan;77(1):128-4
The medical community has long recognized fungi as important allergens for patients with asthma. Interestingly, fungal sensitization is more common in children and has been linked to severe asthma resulting in death. The accepted paradigm is that fungal sensitization occurs as a result of recurrent, transient environmental exposures. Yet, increasing evidence suggests that fungi may interact with people in unrecognized ways to promote asthma. My lab is interested in understanding the role of subclinical fungal infections in asthma and their potential contribution to the high prevalence of asthma in urban areas.

We have demonstrated that the majority of Bronx children older than 2 years have serologic evidence of cryptococcal infection. *Cryptococcus neoformans* is an encapsulated fungus that is well suited to serve as co-factor in urban asthma. *C. neoformans* colonizes pigeon droppings and is endemic to urban areas. Once inhaled, this fungus causes persistent, subclinical infections. Cryptococcal infection induces TH2 inflammation in animal models. In a rat model, we have shown that cryptococcal pulmonary infection acts a co-factor to enhance allergic inflammation to allergen challenge and promotes airway hyper-responsiveness, both hallmark features of asthma. Pulmonary cryptococcosis also induces chitinase expression, which has recently been implicated as an essential mediator of allergic inflammation.

In addition to fungal studies, my lab is interested in anthrax pathogenesis. *Bacillus anthracis* is widely recognized as a potential agent of bioterrorism as evidenced by the 2001 anthrax attack. The toxins of *B. anthracis* are essential to virulence. In collaborations with Drs. Arturo Casadevall and Jurgen Brojatsch, we have studied the mechanisms by which *Bacillus anthracis* toxins contribute to host death. We have identified a previously unrecognized protease in human serum that inactivates the protective antigen component of lethal toxin in vitro. The precise protease and its role in the host response and susceptibility to anthrax remain to be determined. We have also identified a potential role for platelet activating factor (PAF) in mediating the lethal effects of toxin, including the alterations in vascular permeability which is characteristic of anthrax. Together, these observations may have important implications in developing new approaches to the treatment of anthrax.

**PUBLICATIONS**


DR. HARRIS GOLDSTEIN

Our laboratory is utilizing novel approaches to investigate two high priority areas of HIV research, mobilizing the immune system to eradicate HIV infection and evaluating approaches to prevent acquisition of HIV-1 infection. One approach we are using is to stimulate NK cells to suppress acute in vivo HIV-1 infection and eliminate latent HIV-1-infected cells after reactivation. We recently demonstrated that anti-HIV-1 NK cell activity can be markedly augmented by activating them by treatment with an interleukin-15 (IL-15) superagonist, IL-15 bound to soluble IL-15Rα, an approach that potentiates human NK cell-mediated killing of tumor cells. In vitro stimulation of human NK cells with a recombinant IL-15 superagonist significantly induced their expression of the cytotoxic effector molecules and their degranulation. To determine the in vivo capacity of the IL-15 superagonist to activate NK cells to eliminate HIV-1-infected T cells, we used a high-throughput in vivo assay we developed to overcome the inability of mice to be infected with HIV-1, hu-spl-PBMC-NSG mice. This model consists of highly immunodeficient NOD-SCID-IL2rγ(-/-) (NSG) mice intrasplenically injected with activated human PBMCs and challenged by intrasplenic HIV-1 injection. IL-15 superagonist treatment potently inhibited acute HIV-1 infection of hu-spl-PBMC-NSG mice, even when delayed until 3 days after intrasplenic HIV-1 inoculation. Thus, in vivo immunostimulation of NK cells, a promising therapeutic approach for cancer, may represent a new post-exposure treatment to prevent infection HIV-1-infection. We are extending these findings to examine the capacity of activated NK cells to eliminate reactivated latent HIV-1 infected cells as a modality to contribute to eradicating HIV-1 infection. In parallel, we are investigating another approach to eliminate reactivated latent infected cells, utilizing molecular engineering to reprogram the antigen specificity of CD8+ T cells to recognize HIV-specific epitopes and in generate potent HIV-specific CD8+ cytotoxic T lymphocytes (CTLs) by transducing them with lentiviruses expressing the genes encoding the alpha and beta chain of TCRs derived from potent HIV-specific CTLs into CD8+ lymphocytes. In addition, we are examining approaches to increase the cytotoxic capacity of these cells by using lentiviral vectors to express anti-apoptotic genes or cytotoxic genes in these cells. Furthermore, we are investigating the capacity of engineered bispecific antibodies to eliminate reactivated latent infected cells.

We are also using a novel transgenic mouse we developed, hu-CD4/R5/cT1 mice, which circumvents major entry and transcription blocks preventing murine HIV-1 infection by targeting transgenic expression of human CD4, CCR5 and cyclinT1 genes to CD4+ T cells and myeloid-committed cells. These mice develop disseminated HIV-1 infection after intravenous HIV-1 challenge and local HIV-1 infection after intravaginal inoculation. We are utilizing these mice to evaluate the in vivo efficacy of novel HIV-1 vaccines. In addition, we are using these transgenic mice to evaluate the mechanisms by which co-infection facilitates HIV-1 acquisition and to determine the efficacy of different preventive therapies. Epidemiological studies have demonstrated that HSV-2 infection significantly increases the risk of HIV-1 acquisition, thereby contributing to the expanding HIV-1 epidemic. To investigate whether HSV-2 infection directly facilitates mucosal HIV-1 acquisition, we infected the hu-CD4/R5/cT1 mice with HSV-2 and then intravaginally challenged the mice with HIV-1. Co-infection with HSV-2 markedly increased lower genital HIV-1 infection and HIV-1 dissemination to draining lymph nodes. HSV-2 infection stimulated local infiltration and activation of CD4+ T cells and dendritic cells, likely contributing to the enhanced HIV-1 infection and dissemination in HSV-2 co-infected mice. We used this model to demonstrate that a novel gel containing the tenofovir prodrug, tenofovir disoproxil fumerate (TDF), not the tenofovir microbicide gel utilized in the recent CAPRISA 004, VOICE and FACTS 001 clinical trials, was effective as pre-exposure prophylaxis (PrEP) to prevent vaginal HIV-1 infection in HSV-2-coinfected mice. These transgenic mice are also being used to evaluate novel approaches to “cure” HIV-1 infection.
Publications


Our laboratory is focused on the development and testing of cancer immunotherapy and non-immune-based cancer therapies. Since most cancer deaths occur by metastases (primary tumors can often be removed by surgery, chemotherapy, or radiation), our therapies are focused on the treatment of metastases. We have developed various therapies using different novel approaches, in preclinical mouse tumor models with metastatic breast and pancreatic cancer. For instance, we use an attenuated bacterium *Listeria monocytogenes* as a platform for the delivery of anticancer agents to the tumor microenvironment and into tumor cells such as radioactivity, tumor-associated antigens, or small molecules like alphagalactosylceramide, or we kill tumor cells through cryoablation by freezing and thawing tumor cells, combined with various adjuvants targeting myeloid-derived suppressor cells (MDSC) such as stimulator of interferon genes (STING)-ligand cyclic di-guanylate (c-di-GMP, Curcumin, and AMD3100. Since MDSC play a major role in immune suppression in the tumor microenvironment, MDSC are an important target in cancer immunotherapies. We also focus on the age factor since most cancer patients are old and elderly react less efficient to vaccines than young adults. The MDSC are present in blood of patients and mice with cancer. This MDSC population is strongly increased in the tumor microenvironment particularly at older age, and contributes to the age-related T cell unresponsiveness.

**Listeria-based cancer vaccines**

Attenuated *Listeria monocytogenes* is a weakened facultative anaerobic bacterium (non-toxic and non-pathogenic) and has been used to deliver antigens into antigen-presenting cells. We developed various Listeria-based constructs expressing tumor-associated antigens including Mage-b, Survivin, p53 etc and tested these constructs in mice with metastatic breast and pancreatic cancer, and demonstrated a significant reduction in metastases and tumor growth. In addition, we have further improved the efficacy of the Listeria-Mage-b vaccine with help of MDSC-targeting adjuvants like c-di-GMP and Curcumin. However, in 2009 our lab discovered that Listeria infects and kills tumor cells by the generation of reactive oxygen species (ROS) through the activation of the NADPH-oxidase pathway, and left healthy tissues unharmed. Based on this tropism for the tumor microenvironment we started using Listeria as a platform for the selective delivery of anti-cancer agents to the tumor microenvironment. For more detail see Kim et al, Cancer Res 2009; Chandra et al, Cancer Immunology Research, 2014.

**Mechanisms that contribute to the selective survival and multiplication of Listeria in the tumor microenvironment**

We have analyzed potential mechanisms explaining why Listeria survived and multiplied in the TME and not in healthy tissues. We discovered that Listeria is protected from immune clearance in the TME through strong immune suppression, but is rapidly killed in healthy tissues that lack immune suppression. In addition, we found that MDSC play an important role in the selective delivery and survival of Listeria in the tumor microenvironment. MDSC are selectively attracted by the primary tumor through the production of attractants such as granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-6, A100. Listeria infects, survives and multiplies in MDSC of tumor-bearing mice, and is protected from immune clearance because of the immune suppressive character of MDSC. We have shown that Listeria, once at the tumor site, infects (and kills) tumor cells directly or spreads from MDSC into tumor cells through the cell-to-cell spread mechanism specific for Listeria. For more detail see Quispe-Tintaya et al, PNAS 2013; Chandra et al, BJC, 2013.

**Radioactive Listeria for the treatment of pancreatic cancer**

Ninety six percent of patients diagnosed with pancreatic cancer have only 6 months to live, despite aggressive treatments. This underlines the urgent need for new effective therapies. In
collaboration with Dr. Ekaterina Dadachova (Department of Radiation, Einstein), we developed a radioactive Listeria for the treatment of pancreatic cancer, by coupling $^{188}\text{Re}$henium to anti-Listeria antibodies followed by incubation with Listeria bacteria. This resulted in the synergistic destruction of cancer cells through Listeria-induced ROS and through ionizing radiation of the $^{188}\text{Re}$. The number of metastases was reduced by 50% in mice treated with Listeria alone, and by 90% in mice treated with Listeria-$^{188}\text{Re}$. This correlated with the accumulation of radioactivity in the metastases. This was the first time that a live attenuated bacterium was successfully used to deliver radioactivity selectively to the tumor microenvironment. The potential of the radioactive Listeria for the treatment of pancreatic cancer was discussed in several high profile journals like Science, Nature, as well as lay Journals like The Economist, Forbes Magazine and many others. Currently, we are testing Listeria with other radioisotopes. For more detail see Quispe-Tintaya et al, PNAS 2013.

**Listeria incorporated with alphagalactosylceramide**

In collaboration with Dr. Steven Porcelli (Department Microbiology and Immunology, Einstein), we incorporated alphagalactosylceramide (αGC) into the Listeria bacteria simply during culture. This method was originally developed for mycobacteria. αGC is a marine sponge that activates natural killer T cells (NKT) cells, which in turn stimulates other immune cells like natural killer (NK) cells and T cells. We demonstrated that Listeria expressing tumor-associated antigen Mage-b incorporated with αGC created an immune-stimulating environment that attracted the NKT cells to the metastases, resulting in improved activation of CD8 T cells to Mage-b and a dramatic reduction in the number of metastases in a mouse model of metastatic breast cancer (4T1). For more detail see Singh et al, BJC 2014.

**Cryoablation combined with adjuvants**

Cryoablation involves killing of tumor cells through freezing and thawing, resulting in recruitment of tumor-specific T cells. Since MDSC strongly inhibits these T cells we have the cryoablation combined with MDSC-targeting adjuvants like STING ligand c-di-GMP, Curcumin, and AMD3100. c-di-GMP reduces the number of MDSC and converts a subpopulation of MDSC into an immune-stimulating phenotype producing IL-12 (stimulates T cells). Curcumin reduces IL-6 produced by MDSC and breast tumors. IL-6 strongly inhibits T cells in the tumor microenvironment. AMD3100 is a small molecule that prevents the interaction of CXCR4 on MDSC and stromal cell-derived factor (sdf-1) on tumor cells. Currently, these combination therapies are under investigation in mice with metastatic breast cancer in collaboration with the Anticancer Fund (Brussels, Belgium). Preliminary results are extremely promising. Cryoablation and Meriva (Curcumin derivate) significantly delayed the onset of metastases and eliminated completely the primary tumor, prolonged the survival rate compared to the control groups in correlation with improved CD8 T cell responses to multiple tumor-associated antigens. For more detail see Chandra et al, OnolImmunology 2015.

**Feasibility of cancer vaccination at older age**

Cancer is a disease of the elderly. When cancer becomes metastatic, it often needs aggressive second-line treatment, for which the options are limited. This is particularly challenging for frail, elderly cancer patients in which comorbidity plays an antagonistic role. Immunotherapy is the most promising and benign option for preventing or curing metastatic cancer in such patients. Unfortunately, cancer immunotherapy is less effective at old than at young age, due to T cell unresponsiveness, especially in the tumor microenvironment (TME). Various causes have been described for T cell unresponsiveness at old age, such as lack of naïve T cells at older age, deficiency in the upregulation of co-stimulatory molecules on aged dendritic cells (DCs), and most recently, the increase in the population of MDSC in the TME of old compared to young mice, among other age-related immune impairments. As mentioned above, Listeria has an intimate relationship with MDSC. We have shown that Listeria-based vaccination was equally effective in young and old mice with metastatic breast cancer by targeting MDSC. The Listeria
killed the tumor cells directly through ROS, and Listeria-activated T cells killed the infected tumor cells presenting the Listeria antigens. For more detail see Chandra et al, BJC, 2013.

Most recent peer-reviewed publications relevant to the field of cancer vaccination and cancer therapies selected out of 46


Quispe-Tintaya W*, Chandra D*, Jahangir A, Harris M, Casadevall A, Dadachova E, and Gravekamp C. A non-toxic radioactive Listeriaat is a highly effective therapy against metastatic pancreatic cancer. PNAS, 2013; 110(21):8668-73. PMID: 23610422.*Both authors contributed equally to the manuscript.


Most recent invited publications relevant to the field of cancer vaccination at older age selected out of 14


RESEARCH IMPACT


Websites of Journals and magazines that discussed the impact of the radioactive Listeria for therapeutic treatment of pancreatic cancer:

http://www.nature.com/nrgastro/journal/vaop/ncurrent/full/nrgastro.2013.81.html

http://news.sciencemag.org/sciencenow/2013/04/radioactive-microbes-nuke-tumor-.html

http://txchnologist.com/post/48768770496/radioactive-metal-hitches-ride-on-bacteria-to-fight


https://www.sciencenews.org/article/microbes-can-redeem-themselves-fight-disease
key words: HIV-1 uncoating and reverse transcription, restriction factors TRIM5alpha, TRIMCyp, transportin-3 (TNPO3), CPSF6, SAMHD1, MxB, SERINC5, elite controllers, and HIV-1 T cell restriction factors.

My research program is focused on understanding early events of HIV-1 infection such as uncoating, reverse transcription and nuclear import. To this end, we have exploited a group of proteins that are expressed by the host, and block HIV-1 infection at early stages. These proteins, known as restriction factors, have allowed us to understand fundamental processes in the HIV-1 life cycle. Besides assisting the understanding of fundamental problems on HIV-1 biology, restriction factors represent a new frontier in the search for an effective HIV-1 cure. The following sections explain our past findings, ongoing and planned research.

1) HIV-1 Uncoating

HIV-1 uncoating occurs early in infection, and is the shedding of monomeric capsid from the HIV-1 core, which is composed of 1500 monomers of capsid protein assembled into a conical structure containing the RNA viral genome. Our investigations revealed, contrary to an old dogma, that HIV-1 reverse transcription occurs before or during uncoating but not after (Roa et al., 2012)(Fig. 1). Furthermore, we demonstrated that genetic or pharmacological inhibition of reverse transcription inhibits the uncoating process during infection (Yang et al., 2012). These experiments suggested that internal rearrangements inside the core start the uncoating process. In agreement, we found that cytosolic extracts stabilized the HIV-1 core during infection in vivo and in vitro (Fricke et al., 2013a). Overall, our work suggests that HIV-1 cores are stable in the cytosol, and that initiation of uncoating is triggered from inside the core.

[Diagram of HIV-1 uncoating process]

**Figure 1. Current model for the occurrence of HIV-1 reverse transcription and uncoating.**
Our investigations showed that HIV-1 reverse transcription is completed inside the viral core during/before uncoating. This is in stark contrast of a past dogma that suggested that reverse transcription occurs after uncoating.

The study of HIV-1 uncoating in vitro has been hindered by the unstable nature of the HIV-1 core outside the cellular environment. This evidence together with work mentioned above suggests that the HIV-1 core is stabilized by cellular factors. Future work on this area will use biochemical and genetic approaches to identify factors that stabilize the HIV-1 core in the cellular environment. To this end, we will biochemically isolate HIV-1 cores from infected cells and identify the proteins associated to the core by mass spectrometry. We will compare the
protein content of HIV-1 cores stabilized by different conditions: using reverse transcription inhibitors (Yang et al., 2012), viruses containing a defective reverse transcriptase enzyme (Yang et al., 2012), the microtubule disruptive drug nocodazol (Lukic et al., 2014; Malikov et al., 2015), cells expressing cytoplasmic CPSF6(Fricke et al., 2013b), and cells expressing MxB/Mx2(Fricke et al., 2014). As a negative control, we will mock isolate cores from cells expressing rhesus TRIM5α, which accelerates uncoating (Diaz-Griffero et al., 2007a; Perron et al., 2007; Stremlau et al., 2005). The assays we have developed to study capsid stability in vitro and in vivo will be used to confirm these interactions(Fricke et al., 2013a). Finally, the contribution to uncoating and infection will be evaluated in cells where the candidate proteins are knockout using the Cas9/Crispr technology that is already working in our lab. Finding proteins that stabilize the HIV-1 core during infection will provide fundamental understanding on the uncoating process of HIV-1.

2) TRIM5α

The HIV-1 restriction factor TRIM5α is composed of four domains: RING, B-box-2, coiled-coil and PRYSPRY domains (Fig. 2A). To understand the contribution of these different domains to restriction, we have solved the structure of the RING, B-Box-2 and PRYSPRY domains (Biris et al., 2013; Diaz-Griffero et al., 2009; Lienlaf et al., 2011; Roa et al., 2012). Our structure-function studies revealed: 1) the RING domain provides E3 ligase activity, which is necessary for restriction(Lienlaf et al., 2011; Roa et al., 2012), 2) the B-box-2 domain regulates the ability of TRIM5α to form higher-order complexes(Diaz-Griffero et al., 2009; 2007b), which is an essential function for the ability of TRIM5α to form an array of protein in the surface of the core (Fig.2B)(Ganser-Pornilos et al., 2004). 3) the PRYSPRY domain is the domain that comes in direct contact with the HIV-1 core (Yang et al., 2014). In summary our findings suggested that the rhesus macaque protein TRIM5α binds to the surface of the HIV-1 core by forming an array protein (Fig. 2B). Formation of this complex recruits Ubc13, which is an E2 enzyme required for restriction(Pertel et al., 2011). Subsequently, an unknown activity leads to acceleration of uncoating (Diaz-Griffero et al., 2007a; Stremlau et al., 2006). Although, we have recently solved the structure of Ubc13 interacting with the RING domain (Yudina et al., 2015), the mechanism and energy source by which this complex leads to acceleration of uncoating is unknown (Fig.2B).
Figure 2. Inhibition of HIV-1 infection by TRIM5α. (A) The different domains of TRIM5α are shown, and a small cartoon depicting the TRIM5α protein is shown on the right side. (B) TRIM5α proteins assembled forming a hexagonal pattern on the surface of the HIV-1 core, and the RING domain of TRIM5α recruits an E2 enzyme (Ubc13). Subsequently the core is disassembled (acceleration of uncoating) and infection is aborted. The mechanism and source of energy for this process is unknown.

Interestingly, the structure of the PRYSPRY domain, which is the domain that directly interact with the HIV-1 core, exhibit a flexible loop in the region that interacts with the HIV-1 core (Fig. 3), as established by genetic experiments (Li et al., 2006; Yap et al., 2005). These observations suggested that movement of the loop is providing the energy necessary for the complex to accelerate uncoating. Future experiments will test the hypothesis that a flexible loop is required for acceleration of uncoating. To this end, we will identify TRIM5α mutations on the PRYSPRY domain that decrease the flexibility of the loop but preserve binding to the HIV-1 core. These particular variants will be tested for their ability to block HIV-1 and accelerate uncoating in human cells. These studies will be complemented by experiments that will measure the ability of the PRYSPRY domain mutants in solution to disassemble in vitro assembled HIV-1 CA complexes (Fricke et al., 2013a). Overall these experiments will sort out the role of loop flexibility in acceleration of uncoating.
Figure 3. Structure of the PRYSPRY domain of rhesus monkey TRIM5α. The structure of the PRYSPRY domain is shown. The four variable loops of the protein are indicated as V1, V2, V3 and V4. The V1 loop, which directly interact with the HIV-1 core, exhibited hundred of different conformations, as indicated by the strands in different colors. These observations suggested that the PRYSPRY domain of TRIM5α exhibit great plasticity, which might allow the binding to different epitopes on the surface of the HIV-1 core. In addition, the movement of the V1 loop might be the energy necessary for accelerating the uncoating process of HIV-1.

3) MxB/Mx2

The restriction factor MxB is an interferon-a inducible protein that blocks HIV-1 infection in T cells (Goujon et al., 2013; Kane et al., 2013; Liu et al., 2013). Our investigations revealed that MxB blocks HIV-1 infection by inhibiting the uncoating process of HIV-1 (Fricke et al., 2014) (Fig. 4). We found that MxB directly interacts with the HIV-1 core by using a triple arginine in the N-terminal domain of MxB (Schulte et al., 2015). Our studies also showed that oligomerization of MxB is essential for the ability of MxB to bind to the HIV-1 core and restrict HIV-1 (Buffone et al., 2015). Overall, these results suggested that MxB binding to the core is forming an array on the surface of the HIV-1 core. Future experiments will test the hypothesis that MxB forms an array of protein on the surface of the HIV-1 core, which leads to inhibition of uncoating. To this end, we will perform Electron Microscopy of pure MxB protein overlaid on in vitro assembled HIV-1 CA that forms flat sheets. These experiments will show whether MxB cages the HIV-1 core in order to prevent HIV-1 uncoating. We are currently testing our purified MxB protein from human cells for its ability to interact with HIV-1 cores.
Figure 4. Inhibition of HIV-1 by MxB/Mx2. Our investigations revealed that MxB directly interacts with the HIV-1 core and prevents the uncoating process of HIV-1 terminating infection.

4) SAMHD1

The restriction factor SAMHD1 prevents HIV-1 infection of macrophages, dendritic cells, and resting T cells (Baldauf et al., 2012; Hrecka et al., 2011; Laguette et al., 2011). Our investigations revealed that SAMHD1 is regulated by phosphorylation of T592 (White et al., 2013a; 2013b) (Fig. 5). The unphosphorylated form of SAMHD1 potently blocks HIV-1 infection. By contrast the phosphorylated form does not affect HIV-1 infection. These investigations suggested that the ability of SAMHD1 to block HIV-1 infection can be modulated. To this end, we are currently investigating in human primary cells the regulation of phosphorylation by different cytokines. We recently found that SAMHD1 is S-glutathionylated, and that this post-translational modification is essential for the ability of SAMHD1 to block HIV-1 infection. We are currently investigating the contribution of SAMHD1 S-glutathionylation to restriction. Although we have performed extensive biochemical and cellular characterization of SAMHD1 (Brandariz-Nuñez et al., 2013; 2012; Ryoo et al., 2014; St Gelais et al., 2014; Welbourn et al., 2012; White et al., 2013b; 2014), we have not explored the role of SAMHD1 in immunity. Efficient lentiviral infection of macrophages in old world monkeys correlates with a strong adaptive immunity (Schaller et al., 2012).
Figure 5. Regulation of SAMHD1 anti-HIV-1 activity by phosphorylation. Our investigations revealed that phosphorylation of SAMHD1 at T592 modulates the ability of this restriction factor to block HIV-1 infection of macrophages.

We are currently testing the hypothesis that SAMHD1 is involved in adaptive immunity. To this end, we will study adaptive immunity in the SAMHD1 knockout mice by testing antibody response, and the ability of the mice to prevent the growth of diverse pathogens. We will initially test pathogens that are known to be inhibited by SAMHD1, such as HSV-1/2 (Kim et al., 2013) and mycobacterium tuberculosis (our unpublished preliminary studies). These investigations will help us understand the role of SAMHD1 in the immune system.

PUBLICATIONS
(h-index=27, total citation=2618). Total papers = 69


Genital herpes infections are a major global health problem and a substantial co-factor for HIV infection. Development of new approaches to prevent HSV and HIV infection requires an understanding of the molecular and cellular events critical for the establishment of infection. Studies demonstrating that the initial step in HSV infection is binding to heparan sulfate were instrumental in advancing sulfated polymers as candidate vaginal microbicides because they competitively block attachment. Importantly these molecules also block binding of HIV to target cells through interactions with gp120. Several of these drugs have been advanced to Phase III clinical trials. However, studies from our laboratory indicate that some of these drugs have limited efficacy against non-clade B HIV isolates and lose activity when virus is presented in the context of seminal fluid. These findings may have contributed to the failure of several of these drugs in recently completed clinical trials. We hypothesize that prevention will require a multi-targeted approach with drugs directed at different steps in the entry process.

There are several major areas of research in the laboratory. First, a major focus is to identify the signaling pathways required for HSV-2 invasion using cells important in transmission, which include human vaginal and cervical epithelia and immune cells and exploit this knowledge to develop novel strategies for prevention. Current work from our laboratory demonstrates that HSV activates calcium (Ca^{2+}), Akt, integrin, and phosphorylation signaling pathways and that these signaling pathways play critical roles in the establishment of infection. More recent work shows that integrin signaling is also critical for cell-to-cell spread of virus. We use a combination of siRNA, confocal microscopy and other methods to identify cellular pathways harnessed by the virus for entry and then translate this work into a murine model to explore novel therapeutic strategies.

In related studies, we also explore how HSV overcomes mucosal immunity to initiate infection and how the changes triggered in the mucosal environment contribute to the synergy between HSV and HIV. Using a dual chamber culture system, we have shown that exposure of human cells or explant tissue to HSV disrupts tight junctions, leading to a loss in the epithelial barrier and enhanced migration of HIV across the epithelium. Moreover, HSV downregulates adherens junction proteins. In addition, HSV triggers an inflammatory response characterized by activation of NF-κB pathways, release of pro-inflammatory cytokines and chemokines, and downregulates SLPI. We hypothesize that these responses enhance HIV acquisition and replication in the setting of HSV infection.

An additional area of research focuses on defining mucosal immunity in the genital tract as this knowledge could identify novel factors that could be exploited as protective microbicides and should facilitate development of biomarkers of microbicide safety. We previously showed that cervicovaginal secretions obtained from healthy women protect against HSV infection and substantially reduce viral yields. This protective activity is reduced among HIV-infected women. Mechanistic studies suggest that this endogenous activity is mediated in part by defensins with contributions from secretory leukocyte protease inhibitor (SLPI) and antimicrobial proteins. Ongoing studies are using proteomic approaches to identify known and novel factors that contribute to this innate defense. Currently, we are expanding these studies and conducting clinical trials to evaluate mucosal immunity in the genital tract in cycling and non-cycling women, focusing on the anti-HSV, anti-HIV and anti-bacterial activity found in genital secretions from healthy women, pregnant women, and HIV-infected women. We are also developing novel assays to evaluate microbicide safety including a dual-chamber culture model, murine and cotton rat models, and alternative clinical Phase I studies.


Tuberculosis, caused by *Mycobacterium tuberculosis*, causes one in four avoidable deaths in the Third World and kills more adults than malaria, AIDS, and all tropical diseases combined. In recent years, the epidemic of TB has collided with the epidemic of HIV causing the TB/HIV Syndemic. There have been dramatic increases in the numbers of new cases, particularly in sub-saharan Africa- one of the consequences of the AIDS epidemic. In addition to these increasing incidences, there has been an emergence of *M. tuberculosis* strains that are resistant to two to all ten of the anti-tuberculosis drugs. This represents a significant problem as drug sensitive TB is difficult to treat as it requires the use of four drugs over a six month time interval (short-course chemotherapy). Tuberculosis caused by strains resistant to the two front-line TB drugs, Isoniazid and Rifampicin, will require a minimum of two years of treatment to cure. Alarmingly, there have emerged TB caused by strains that are resistant to four (Extensively Drug Resistant TB) to all ten (Totally Drug Resistant TB). My lab is focused on understanding the biology of *M. tuberculosis*, particularly in the context of the infection of mammals. We pursue this knowledge of biology with the hopes of developing more effective TB drugs, more effective TB vaccines, and improved diagnostic tests to address drug susceptibilities. Our focus over the last 25 years has been to develop previously unavailable genetic tools and methodologies to gain the knowledge of virulence and drug resistance mechanisms. Currently, the projects in the lab include 1) Generation of fluoromycobacteriophages to identify drug resistant and drug tolerant *M. tuberculosis* cells 2) Identification of the genes that are required to become drug resistant or drug tolerant, 3) Identification of the genes required to evade humoral host responses, 4) Characterization of metabolomics of the cells that are tolerant to TB drugs, 5) Development of high-throughput specialized transduction system to generate precisely defined null-mutants of every gene of *M. tuberculosis*. More recently, in collaboration with Dr. Betsy Herold, we have developed a novel vaccine vector to prevent herpes viruses and other mucosal diseases.

**PUBLICATIONS**


INI1/hSNF5 is a component of the chromatin remodeling SWI/SNF complex. It is an interacting partner for HIV-1 integrase (IN) and also a tumor suppressor biallelically mutated in rhabdoid tumors, a rare but highly aggressive pediatric malignancy. The two major areas of focus in the laboratory are: (i) understanding the role of INI1 in HIV-1 replication and exploring its potential as a drug target for intervention of AIDS; and (ii) understanding the mechanism of tumor suppression by INI1/hSNF5 and developing novel and effective therapeutic strategies for rhabdoid tumors.

INI1 in HIV-1 replication: We have found that INI1/hSNF5 directly binds and recruits components of Sin3a-histone deacetylase (HDAC) complex into the HIV-1 virions and this HDAC1 complex appears to be required for viral infectivity. We are currently isolating and characterizing IN and INI1 mutants defective for binding to HDAC1 complex and testing their effect on HIV-1 replication. We have found that HIV-1 harboring IN mutants defective or binding to INI1 are severely compromised for replication. Furthermore, we have found that IN1 mutants defective for binding to HDAC1 complex dominant negatively inhibit HIV-1 but not SIV replication. These studies are likely to open up a new paradigm for role of INI1 in HIV-1 replication and may provide novel strategies to inhibit viral replication.

Mechanism of Tumor suppression by INI1/hSNF5: By using a series of genetic systems developed in our laboratory and by isolating cancer-associated mutations of INI1, and a wealth of protein-protein interaction defective mutants of INI1, we are dissecting the exact mechanism of INI1-mediated G0/G1 cell cycle arrest, mitotic arrest, and senescence and tumor suppression. Furthermore, characterizing the INI1-associated HDAC1 complex has revealed an unanticipated role of INI1 in interferon signaling and tumor suppression.

Development of targeted therapies for rhabdoid tumors based on INI1 function: One of the goals of our laboratory is to develop molecularly targeted therapies based on the understanding of genesis of rhabdoid tumors. Majority of rhabdoid tumors have biallelic inactivation of INI1 gene. Our previous studies demonstrated that Cyclin D1 is a direct downstream target of INI1 mediated repression and that rhabdoid tumors are exquisitely dependent on Cyclin D1 for genesis and survival. Our preclinical studies have provided proof of principle for our hypothesis that targeting Cyclin/cdk axis is an effective means of inhibiting rhabdoid tumors in vitro and in vivo. The current goal is to develop novel strategies to facilitate clinical translation of laboratory findings to establish an effective therapy for these tumors. For this purpose, we are using non-invasive imaging technology such as microPET to monitor the therapeutic efficacy in primary mouse tumor models, developing novel drugs to target these tumors and investigating the interaction between Cyclin D1, the cdk pathway and Ini1 in mouse models.

Identification of downstream pathways regulated by INI1 has been instrumental in novel biomarkers and therapeutic targets for these tumors. Aurora A is repressed by INI1 and it is de-repressed in rhabdoid tumors due to loss of INI1. We have found that Aurora A is a novel therapeutic target as siRNA-mediated depletion of this gene resulted in potent mitotic catastrophe and cell death in rhabdoid tumors.

PUBLICATIONS:


Our lab is at the forefront of microbial ecology, studying the interplay between diversity and functional capacity in natural microbial communities.

We focus on: 1) the capacity for microbes to metabolize excreted drugs in human populations, and 2) the influence of mobile element pools on microbial ecosystem functional capacity.

Microbial genomes harbor tremendous diversity at the gene level even within closely related taxonomic groups. Microbes exchange DNA, with each other and with viruses, and can also take up DNA from the environment, leading to variability in the functional capacity of individual cells. Microbial ecosystems, therefore, are a social network of interacting and mobile genes with the capacity for tremendous functional plasticity. Microbes in the gut carry enzymes with the potential to metabolize excreted drugs, some of which cause adverse drug responses in patients. We study the abundance and phylogenetic distribution of microbial enzymes in human guts to predict the capacity of patients to metabolize drugs. Our focus is on the question: what forces enable genetic mobility, or information flow, in microbial ecosystems and how do these forces contribute to the evolution of community functions?

We work with microbial communities from marine systems and the human gut. The specific goals of the lab are to develop a pharmacokinetics of the human microbiome by incorporating the many enzymes with the potential to interact with excreted drugs; and to predict gene mobility and spread to enable targeted manipulation of the metabolic capacity of microbial communities in diverse environments.

**PUBLICATIONS**

For a complete list see: https://scholar.google.com/citations?user=sg7-rm4AAAAJ&hl=en

† indicates authors contributed equally to the work


B. Li†, D. Sher†, **L. Kelly**, Y. Shi, K. Huang, P. J. Knerr, I. Joewono, D. Rusch, S. W. Chisholm, and W. A. van der Donk, “Catalytic promiscuity in the biosynthesis of cyclic peptide secondary


Toxoplasma gondii and Plasmodium species affect over one third the world’s population. These parasites are members of the Apicomplexa phylum, obligate intracellular parasites that are common human and veterinary pathogens. Plasmodium species cause malaria, which affects an estimated 500 million people per year and causes 1 million deaths per year. T. gondii is an important cause of birth defects and opportunistic infections in the immunocompromised including AIDS patients and is a Category B biodefense agent. Our research activities have been devoted to understanding the mechanisms by which these parasites are able to survive within host cells and cause disease. We use a variety of molecular biology, cell biology and genetic techniques to understand the pathogenesis of parasitic infections. Recently we have also begun efforts to understand how host gene expression and host factors impact the pathogenesis of parasitic infections.

Current projects include:

1. **Systems biology of T. gondii host-pathogen interactions.** Most clinical toxoplasmosis is due to reactivation of the latent bradyzoite form that persists throughout the life of an infected host. The ability to switch from the tachyzoite form to the latent bradyzoite form is central to disease pathogenesis and is regulated by both genetic and epigenetic factors. We are using a multidisciplinary approach using genomics, proteomics, cell biology and bioinformatics to understand the mechanisms by which T. gondii parasites regulate gene expression and sense changes in their environment.

Many parasites have involved clever ways to evade host defenses and alter host gene expression to create a more hospitable environment. Using the tools we have developed in our study of T. gondii epigenomics, we are examining how T. gondii alters human gene expression and the human epigenome, and the effects of these changes on human disease.

2. **Impact of HIV coinfection upon cerebral malaria.** Cerebral malaria is a major cause of death from malaria, but the molecular mechanisms of death and pathogenesis are not understood. It is now appreciated that HIV significantly affects the course and severity of malaria infection. We are trying to understand how HIV and malaria interact to exacerbate the severity of cerebral malaria using an in vitro blood brain barrier model, a mouse model of HIV and cerebral malaria and pathological studies in human samples.

**PUBLICATIONS**


Hochman SE, Madaline TF, Wassmer SC, Mbale E, Choi N, Seydel KB, Varughese J, Grau GER, Kamiza S, Molyneux ME, Whitten R, Taylor TE, Lee S, Milner DA, **Kim K.** Fatal Pediatric Cerebral Malaria is Associated with Intravascular Monocytes and Platelets that are Increased with HIV Co-infection. mBIO In Press 2015
Immune effector cell differentiation & protective host responses against microbial pathogens in vivo

Microbial pathogens invasion usually triggers potent host immune responses, however efficient protection and pathogen killing require the presence of effector cells and combinations of inflammatory signals that are ill-defined in most infections.

Our work therefore focuses on precisely defining these events in vivo. Specifically, we investigate (i) the inflammatory signals and related pathways, and innate immune cells that regulate T cell differentiation, and (ii) the cross-talks between memory T cells and innate immune cells during recall infections. Innate immune cells include monocytes, macrophages, dendritic cells and lymphocytes. We also use various models of acute infections in mice, namely the bacteria Listeria monocytogenes, Streptococcus pneumoniae, the viruses Vesicular Stomatitis virus and Murine Cytomegalovirus and the parasites Plasmodium yoelii and Toxoplasma gondii. We take advantage of a range of advanced fluorescent-tracer based methodologies to monitor immune cells in situ. We use cell transfer experiments and novel genetically modified mice models in which dynamic cell functions can be monitored and/or in which functional subsets of immune cells can be selectively eliminated.

Overall, the goal of my laboratory is to improve our fine understanding of the factors that orchestrate antimicrobial host protective immune responses in vivo. We believe that our work will contribute to better immune cell-mediated preventive and therapeutic vaccination strategies.

PUBLICATIONS


Soudja M'Homa S., Ruiz A. L., Marie J. C., and G. Lauvau. Inflammatory monocytes switch on memory CD8\(^+\) T and innate NK lymphocytes during microbial pathogens invasion. *Immunity*, accepted.
My laboratory is focused on understanding the molecular basis of life. Our interdisciplinary pursuit of this issue has provided a broad experimental platform for our work and has proven a recipe for discovery. For example, my group discovered the link between sulfur biology and GTPase function; a linkage that rests with the enzyme ATP sulfurylase, which allosterically couples the chemical potential of GTP hydrolysis to the synthesis of activated sulfate (APS, adenosine 5'-phosphosulfate), an essential sulfur metabolite. Our inquiries in this area have revealed further that this same enzyme forms a complex with its partners in the cysteine biosynthetic pathway, and, remarkably, that new catalytic function emerges from this complex - the hydrolysis of ATP. In this case, it is the energy of ATP hydrolysis that is linked to the synthesis of APS. This finding underscores how cellular components can combine in synergistic ways to create hierarchies of function. Such hierarchies, whose behaviors are rooted in the reduction of entropy, are not well understood, and are of keen interest to us. First principles of chemistry and enzymology suggested that ATP sulfurylases that are not linked to an external energy source, such as ATP or GTP hydrolysis, might transfer APS directly to the active site of the next enzyme in the metabolic pathway, APS kinase. We have shown that in a spectacular display of the interplay of structure and function, certain sulfate activating complexes transfer APS directly between the active-sites of ATP sulfurylase and APS kinase via a 75Å-long groove that opens and closes in response to the position of the nucleotide within the groove.

Transfer of the sulfuryl-moiety (−SO₃⁻) from activated sulfate to biological acceptors is used widely by the cell to regulate metabolism, and the extent to which a particular metabolite is sulfated is determined by the balance of the in-vivo activity of the sulfotransferase (which transfers the sulfuryl-group) and sulfatase (which hydrolytically removes it). Compelling, disease-relevant biology pivots on the activities of each of the six known cytosolic sulfotransferase isozymes. Our laboratory has concentrated primarily on estrogen sulfotransferase (EST), which sulfates estrogen and thereby prevents it from binding to and activating the estrogen receptor. Aberrant sulfation of estrogen is tightly, causally linked to cancer in primary estrogen-dependent breast tumors. We have recently determined the first transition-state structure of an enzyme catalyzed sulfuryl-transfer reaction – that of EST. While it is quite gratifying to “see” precisely how the electronic structure of the bonds involved in the transfer reorganizes as enzyme-bound substrate moves between their ground- and transition-states, the structure is also of considerable practical value in that it defines the target for the design and synthesis of sulfuryl-transfer transition-state inhibitors (a perfect transition-state mimic is expected to inhibit with picomolar affinity).

*Streptococcus pneumonia*, a multiple-drug resistant organism, is estimated to take the lives of 3600 people daily, the majority of whom are children and the elderly. We discovered recently that mevalonate kinase, an essential enzyme in the isoprenoid biosynthetic pathway in *Streptococcus pneumoniae*, is potently allosterically inhibited by diphosphomevalonate (DPM), a downstream intermediate in the pathway, and that the human isozyme is not. Genetic and animal studies of this multiple-drug resistant organism have taught us that the mevalonate pathway is essential for the survival of *S. pneumoniae* in the lung, and human serum. Consequently, we have undertaken a major research effort to develop novel antibiotics that target these enzymes in gram-positive bacteria. The program, carried out under the auspices of the NIAID, brings together an interdisciplinary team of faculty, postdoctoral fellows and graduate students in the areas of high-resolution NMR-spectroscopy, crystallography, synthetic chemistry, and biochemistry to explore fundamental issues of allostery, catalysis, and inhibition in these systems. I am pleased to report that our recent efforts have produced inhibitors that act with nanomolar affinity at each of multiple points in the pathway, and are capable of killing infectious *S. pneumoniae* in rich media at ~ 25 μg/ml.

The three projects outlined above comprise the core of our research activities; however, we are also expanding into two new areas. The etiology of how *M. tuberculosis* emerges from
dormancy in the lung tubercle is not yet well understood. We are beginning to define the molecular logic of this transition with Prof. John Chan, an expert mycobacteriologist who has isolated a mutation that activates growth by derepressing dormancy. While the protein that harbors this mutation has not yet been assigned molecular function, we now know that it co-purifies with one-equivalent of adenine nucleotide bound to it, that it slowly hydrolyses ATP, and that its sequence identifies it as a possible element of a signaling network – we are currently testing this hypothesis using genetic and biochemical assays. In a final program, we are collaborating with a single molecule spectroscopist, and biological chemist at Columbia University to better understand and control the ribosomal editing functions of the protein-synthetic machinery toward mis-acylated tRNA. Editing protects against disease by recognizing and rejecting misacylated tRNA; controlling editing gives way to regiospecific incorporation of non-natural amino acids into proteins, which will facilitate myriad scientific endeavors including single-molecule exploration of the cellular milieu.

PUBLICATIONS


Key Words: fungus, histoplasmosis, cryptococcosis, candidiasis, melanin

- **Histoplasma capsulatum**: Research on this fungus pertains to the development of active and passive immunotherapeutics. Since individuals with severe histoplasmosis [such as AIDS patients with disseminated disease] often lack effective cell-mediated immune responses, induction of an effective humoral response or passive administration of antibody has tremendous therapeutic potentials. My laboratory is the first to identify protective monoclonal antibodies for the treatment of *H. capsulatum* infection. We are studying the mechanisms of antibody efficacy and are testing the recombinant antigens as a potential prophylactic and therapeutic vaccine. Also, we have embarked with the Nathenson and Almo labs to study the impact of co-stimulation on histoplasmosis and have demonstrated that the PD-1/PDL pathway is critically important to disease pathogenesis. Antibodies can interfere with this negative co-stimulation pathway and prevent lethal histoplasmosis.

- **Candida parapsilosis**: This is the newest fungus to the laboratory. The incidence of *C. parapsilosis* infections has exploded in recent years and very little is known about its virulence. We developed the first efficient method for targeted gene deletion for *C. parapsilosis* and are actively pursuing targets to define what makes this fungus pathogenic, with a particular emphasis on secreted hydrolytic proteins and lipid metabolism. We are also targeting virulence associated genes in *C. albicans* and studying their role in pathogenesis.

- **Cryptococcus neoformans**: We are primarily using this pathogen to elucidate the impact of melanin in pathogenic fungi. Melanin is a complex polymer of unknown structure that is prevalent throughout the biological kingdoms. Ongoing investigations are examining mechanisms of melanin synthesis and rearrangement by molecular, physical, and immunological methods. This work is in collaboration with Dr. Arturo Casadevall, Departments of Microbiology & Immunology and Medicine.

Additional areas of special emphasis:

**Methamphetamine**: This drug has increasingly become a major scourge on our society. Although the behavioral impact of methamphetamine is well understood, there is a dearth of data on the effect of the drug on immune function. We have established that methamphetamine significantly adversely regulates diverse aspects of immunity. We have an ongoing program to further elucidate the mechanisms and impact of this disregulation.

**Nitric oxide releasing nanoparticles**: We are exploring the therapeutic potential of this novel compound for the treatment of diverse infectious diseases, including bacterial and fungal diseases. This work is in collaboration with Dr. Joel Friedman, Departments of Medicine and Physiology & Biophysics.

**PUBLICATIONS**


RNA interference (RNAi) is a mechanism of gene silencing originally described in plants and invertebrates and more recently in mammalian cells. Double-stranded RNAs are cleaved into 21-25mer duplexes, termed small interfering (si)RNAs, and these siRNAs act as a guide to cleave homologous target mRNA, resulting in a sequence-specific decrease in mRNA. Introduction of siRNAs into mammalian cells leads to degradation of specific mRNA.

RNAi has become a valuable tool in gene discovery as well as an attractive therapeutic candidate. We have shown that intravaginal application of siRNAs targeting viral genes protects mice from a lethal herpes simplex virus (HSV-2) infection.

These data indicate that siRNAs could be used in a therapeutic setting such as preventing a sexually transmitted disease, e.g. HSV-2. However, this work is in its initial stages and many questions remain: Is the siRNA silencing we observe optimal? What determines duration or efficacy of siRNA silencing? Are there off-target responses associated with the siRNA treatment?

One aim of our lab is to address these questions. For example, we will design constructs that will deliver siRNAs in various ways. By comparing the ability of these reagents to confer specific gene knockdown, we hope to gain an understanding of what components are required to achieve optimal silencing.

A second aim is to utilize a hypothesis-based approach to dissect immune responses in various mouse models using siRNAs. By understanding the immunity associated with infection by a particular pathogen we can potentially adjust immune responses to favor pathogen eradication. In this way, siRNAs could also be used as components in a microbicid, either as an alternative or adjunct to siRNAs targeting pathogen-expressed genes at the infection site.

Lab Webpage: http://palliserlab.googlepages.com/

**PUBLICATIONS:**


**DR. LISE-ANNE PIROFSKI**

The focus of the Pirofski laboratory is on antibody and B cell immunity to encapsulated microbes using *Streptococcus pneumoniae* (Pneumococcus) and *Cryptococcus neoformans* (Cryptococcus) as examples. Both of these microbes cause disease in normal and immunocompromised people, particularly those with HIV infection, AIDS, B cell and antibody defects. The laboratory conducts translational studies of the serological, cellular, and molecular response to these microbes in normal and immunocompromised patients and basic scientific studies of microbial pathogenesis and host-microbe interaction. The goals of this research are to understand how innate and acquired antibody and B cell immunity to these microbes confers resistance to disease and to translate this knowledge into novel approaches to treatment and prevention of pneumococcal and cryptococcal disease.

**PUBLICATIONS**


Improving T cell responses for vaccination and disease prevention

Our laboratory studies the control of acquired immune responses by T cells, which we view as the master regulators and key effectors of host defense and immune tolerance. In broad terms, our research can be divided into two interrelated areas. The first is to understand the role of regulatory T cells, with particular emphasis on the activities of a specialized T cell subset known as CD1d-restricted NKT cells. These T cells have the highly unusual property of responding to specific glycolipid antigens, and we are studying ways to control their regulatory functions in various mouse models of disease. The second second major research area is the study of T cell responses against pathogenic microorganisms, especially Mycobacterium tuberculosis. We have recently made significant progress in understanding how mycobacteria block effective host T cell responses, and we are now working to incorporate our findings into the rational and intelligent design of a new tuberculosis vaccine. In the short term, we hope to broaden our understanding of how organisms like M. tuberculosis successfully evade eradication by the immune system. Our major long term goal is to create a genetically or chemically modified live attenuated M. tuberculosis strain that will be safe and effective as a vaccine against tuberculosis.

PUBLICATIONS


Research in our laboratory is focused on three areas of HIV/AIDS: HIV associated neurocognitive disorders (HAND), HIV replication mechanisms and RNA aptamers targeted to HIV.

**HIV associated Neurocognitive Disorders (HAND):** The severe form of HAND, the HIV associated dementia (HAD), is common among clade-B HIV-infected individuals in the US, but less common among individuals infected with clade-C HIV-1 in India, suggesting clade-specific differences in neuropathogenicity. Understanding clade-specific determinants of neuropathogenesis may shed light on the disease mechanism and help develop targeted drugs for HAD. We previously demonstrated that due to a C31S polymorphism, clade C Tat lacks the chemokine function of Clade B Tat that plays a crucial role in an increased brain infiltration of monocytic phagocytes in HAD. We studied neuropathogenesis induced by two HIV-1 clades B and C using SCID mouse HIV encephalitis (SCID-HIVE) model and reported that while the introduction of clade B HIV-1\textsubscript{ADA} into SCID mouse brain recapitulates the key features of human HAD disease, mice exposed to similar inputs of HIV\textsubscript{Inde-C1} (clade C) made fewer memory errors than those exposed to HIV-1\textsubscript{ADA} (clade B). HIV-1\textsubscript{ADA} also caused greater astrogliosis and loss of neuronal network integrity.

Work from many groups has shown that clade C HIV-1 in Southern Africa can induce HAD at much higher incidence than in India. We hypothesized that such variation is due to polymorphism in the neuropathogenesis determinants in Tat or gp120, the two major neurotoxicity determinants of HAND. With respect to Tat, we observed that the percentage of HIV isolates with dicysteine motif in Tat is 2-3% on the Indian subcontinent while in the Southern African countries, they ranged from 19-26%. These data broadly correlate with the HAD frequencies reported from India, South Africa and Botswana (3-4%, 25% and 38% respectively). This finding has been corroborated using a Zambian HIV-1C isolate that displays a C31 residue and thus an intact dicysteine motif. Our in vitro and SCID-HIVE results clearly indicate that Tat dicysteine motif determines neurovirulence. If confirmed in population studies, it may be possible to predict neurocognitive outcomes of individuals infected with HIV-1C by genotyping Tat.

Since Tat is not the only neurovirulence determinant in HIV-1, we examined whether gp120 exhibits intra-clade differences between India and Southern Africa. Our findings indicate that gp120 can also display region-specific differences. For example, the Southern African HIV isolates appear to contain more robust neurovirulence determinants than those in the Indian isolates. Thus, two different viral genes in India appear to show determinants of low neurotoxicity. These results suggest that clinical studies studying the incidence of HAD or HAND to correlate viral genetic differences must examine both Tat and gp120. *Ongoing work in our laboratory is attempting to identify the neurovirulence signatures of gp120 in clade C and clade B virus isolates and exploring the role of exosomes in neurovirulence.*

**Anti-HIV RNA aptamers hematopoietic gene therapy:** We previously developed and tested the efficacy of novel, anti-HIV-1 RNA aptamers to inhibit HIV-1 replication. Aptamers are sequences isolated by the iterative process of SELEX and are highly specific to their targets. The most efficacious aptamers identified in our laboratory as well as combinations of them could be tested in nonhuman primates (macaques). We will introduce such aptamers into hematopoietic stem cells, which will then be used in bone marrow transplantation followed by challenge with chimeric, pathogenic SHIVs. We have thoroughly characterized anti-RT aptamers and generated aptamers to HIV-1 Gag MA and NC proteins. Perturbation of HIV-1 Gag and viral RNA interaction using anti-Gag aptamers has provided new insights showing that preventing Gag-RNA binding causes down-modulation of viral RNA thus inhibiting virus
production. Most recently, we developed high affinity aptamers (Kd = 1nM) to HIV-1 protease. **We are currently characterizing the Nef aptamers to understand the specific Nef functions in HIV replication that are affected by each aptamer.**

**HIV Replication Mechanisms:** We have a long-standing interest in elucidating the mechanistic basis of key steps in HIV-1 replication. In earlier work, we delineated the determinants of polymerase processivity, fidelity of DNA synthesis and strand displacement synthesis by HIV-1 RT. **Our current work is focused on the role of beta chemokines in HIV-1 budding.**

**PUBLICATIONS**

**HIV Neuropathogenesis**


**RNA aptamers targeted to HIV-1**


HIV-1 Replication Mechanisms


Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease that typically affects women in their reproductive years. Involvement of the kidneys, or lupus nephritis, appears in about 50% of lupus patients during the course of their disease. Despite medical treatment, morbidity and mortality from renal disease are common in lupus patients. The overall increase in the incidence of lupus and in the number of deaths from the disease reported in the United States are additional reasons for significant concern.

Anti-double stranded (ds) DNA antibodies are a serologic hallmark of patients with SLE. In recent years it has been increasingly clear that not only are anti-dsDNA antibodies an important diagnostic marker for lupus, but that these autoantibodies are also instrumental in the pathogenesis of lupus nephritis. The mechanisms by which anti-dsDNA antibodies induce renal injury, however, are not completely understood. It has been suggested that anti-dsDNA antibodies bind DNA in the circulation followed by non-specific deposition of these immune complexes in the kidney, or that in-situ immune complexes are formed in the kidney by binding of anti-dsDNA antibodies to nuclear antigens deposited on the glomerular basement membrane. Alternatively, some anti-dsDNA antibodies may cause injury by penetrating into living cells and affecting unidentified metabolic pathways. Finally, we and others have generated evidence that strongly suggests that at least some anti-dsDNA antibodies are pathogenic not by virtue of their affinity for DNA, but rather by direct cross-reactivity with renal antigen.

The long-term goals of the laboratory are to study the antigenic triggers and renal pathogenicity of anti-dsDNA antibodies. We want to understand which antigen(s) can trigger pathogenic anti-dsDNA antibodies, and whether protein antigens can induce a lupus-like anti-DNA response. We are determining the cross-reactive kidney antigen bound by anti-DNA antibodies in human lupus and in mouse models of the disease to understand what determines the nephritogenic potential of these antibodies. Understanding the renal pathogenicity of cross-reactive anti-dsDNA antibodies by identifying the target antigen for these antibodies in the kidney would improve our understanding of a key manifestation of lupus, and would facilitate the development of serological tools to better predict the onset and severity of renal involvement in patients with SLE. Furthermore, identification of the triggering and/or target antigen in lupus will allow us to develop novel approaches to the treatment of lupus, by blocking the effects of anti-DNA antibodies on target organs or by specifically tolerizing pathogenic B cells.

**PUBLICATIONS**


Our laboratory has been involved in the molecular analysis of MHC class II-restricted antigen processing and presentation for the last 15 years. We are focused on the characterization of both endogenous and exogenous antigen pathways. As part of our analysis of exogenously delivered antigen we recently mapped the human proteome and peptidome carried by the human lymph. The peptidome, generated by physiological tissue catabolism, and transported by the pre-nodal lymph, is distinct from the self-peptidome generated in the endosomal compartment. Importantly, unlike self antigen processed by local or nodal antigen presenting cells, which produce epitopes constrained by the endosomal processing activity, self antigens present in the lymph are derived from a wider variety of processing pathways; thus expanding the tissue-specific self-repertoire available for the maintenance of immunological tolerance. A second line of ongoing research involves the characterization of the transport of endogenous antigen from the cytosol to the late endosomal compartments by autophagy. In particular we recently characterized the intersection between microautophagy and the ESCRT system as a way to deliver cytosolic antigens to the inner vesicles of multivesicular late endosomes for antigen processing and MHC class II loading. Finally, a third line of research involves the molecular analysis of dendritic cells and MHC class II-restricted immune responses in immune senescence. We recently discovered that dendritic cells generated from aging bone marrow present an extensively oxidized, glycated and lipoxidated proteome that interferes with processing and presentation of MHC class II restricted exogenous and endogenous antigens.

PUBLICATIONS


Our laboratory focuses on new pathways and immunotherapies of T cell costimulation and coinhibition. We have recently discovered new members of the T cell costimulatory/coinhibitory B7 family and CD28 family, including B7x, HHLA2 and TMIGD2, and are using a variety of experimental approaches (gene knock-out mice, transgenic mice, monoclonal antibodies, crystal structure, etc) to understand how new B7/CD28 family members regulate T cell activation and tolerance. Current emphasis in the lab is placed in the following areas:

1) Novel drugs development: Translational medicine of T cell costimulation and coinhibition;
2) In vivo functions of new B7/CD28 pathways;
3) Human cancer-associated new B7/CD28 pathways and cancer immunotherapy;
4) Roles of new B7/CD28 pathways in autoimmune diseases and immunotherapy;
5) Relationship between new B7/CD28 pathways and infection;
6) Functional and structural characterization of new members of the Ig superfamily.

Our goal is to elucidate the mechanisms by which costimulation and coinhibition regulate T cells in peripheral non-lymphoid organs, and to translate the lessons learned in these studies towards developing new therapeutic strategies for immune-mediated diseases such as cancers, autoimmune disorders, infectious diseases, and transplantation rejection. Our research have won extensive attention from biopharmaceutical industry including some of the biggest drug companies.

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