



**UCLA AIDS INSTITUTE  
and  
UCLA Center For AIDS Research**

**PRESENTS  
THE 2<sup>ND</sup> ANNUAL AIDS RESEARCH SYMPOSIUM**

**“What’s New? Reports from a  
New Generation of UCLA AIDS  
Researchers”**

**Monday, October 19, 2009**

**9:00am to 1:30pm**



**Ronald Reagan UCLA Medical Center Auditorium**

*(B Level, Room B130, 757 Westwood Plaza, Los Angeles, CA 90095)*

**PROGRAM**

- 9:00am Welcome -Irvin S.Y. Chen, Director, UCLA AIDS Institute
- 9:10am David Jesse Sanchez, MIMG, *“Modulation of the Type I Interferon Response by HIV Infection”*
- 9:30am Marta Epeldegui, MIMG, *“Role of direct activation of B cells by CD40L in HIV virions in vivo in AIDS-NHL development”*
- 10:00am Jesse L. Clark, Infectious Diseases, *“Routine Screening for Acute and Recent HIV Infection in Lima, Peru”*
- 10:25am Risa Hoffman, Infectious Diseases, *“Impact of Antiretroviral Regimen and Duration on Risk of Mother-to-Child HIV Transmission in Johannesburg, South Africa”*
- 10:45am to 11:00am Break
- 11:00am John K. Williams, Psychiatry & Biobehavioral Sciences, *“Exploring Childhood Sexual Experiences and Vulnerability to Intimate Partner Violence among African American MSM/W”*
- 11:25am Chandra L. Ford, Public Health, *“An Evaluation of ‘Critical Race Theory and HIV/AIDS Disparities: A Multidisciplinary Think Tank”*
- 11:50am Andrew Levine, Neurology, *“Different Neurocognitive Outcomes in HIV+ Abstinent Cocaine versus Methamphetamine Users”*
- 12:15pm Debika Bhattacharya, Infectious Diseases, *“Women Experience Higher Rates of Adverse Events During HCV Therapy in HIV Infection”*
- 12:35pm to 1:30pm LUNCH

# Basic and Translational Research Presentations

-  David Jesse Sanchez, MIMG, “*Modulation of the Type I Interferon Response by HIV Infection*”
-  Marta Epeldegui, MIMG, “*Role of direct activation of B cells by CD40L in HIV virions in vivo in AIDS-NHL development*”

# Modulation of the Type I Interferon Response by HIV Infection

David Jesse Sanchez

MIMG

While the adaptive immune responses to HIV infection have been extensively studied, less is known about how the innate immune system recognizes and responds to HIV infection. As with any other infection, HIV must also interact and deal with the innate immune system of its human host to grow thus may have ways to antagonize host innate immune responses. This innate immunity is a highly advanced, early-warning system that allows for rapid response to HIV infection – and thus presents itself as a critical system to understand and harness, as novel vaccines and therapeutics are developed against HIV. More specifically the Type I Interferon response is a cellular anti-viral bioshield system that directly blocks the spread and replication of viruses. As such Type I Interferon represents a highly attractive target to focus studies of HIV and innate immunity. Previously, we have determined that there are specific regions of herpes viral genomic DNA that give it a unique structure in the cellular milieu thus acting as an inducer of Type I Interferon. This moves from a paradigm of general synthetic ligands to a more distinct natural, pathogen component that induces innate immunity. We have applied this hypothesis to HIV infection. Contrary to most other viral infections, HIV is unable to induce Type I Interferon release upon infection of its natural reservoir of infection, the T cell. This was surprising especially as we see that lysate of HIV virions is able to induce Type I Interferon when transfected into cells. Additional studies have identified a specific RNA sequence motif in the U5 region of the HIV genomic RNA as a potential natural innate immune inducing ligand of HIV that is directly bound by retinoic-acid-inducible gene I (RIG-I), a host intracellular pattern recognition receptor, that triggers innate immune responses such as Type I interferon induction. Though HIV RNA can induce Type I Interferon, the conundrum remained of why HIV infection did not induce Type I Interferon. We went on to find that HIV seems to potently block RIG-I signaling by degradation of CARDIF (also known as IPS-1, MAVS, or VISA), the RIG-I adaptor protein as infected cells have specific loss of this protein. This effectively blocks RNA induction of Type I Interferon within infected cells. Consistent with these findings, we have also identified two HIV proteins, Vpu and Nef that can strongly antagonize CARDIF by degradation of the protein. Based on these data, we hypothesize that host cells are able to sense HIV infection by recognizing specific ligands in the HIV genome and generate an appropriate innate immune response. However, as a pathogen that has evolved within its host, HIV has developed effective strategies such as Vpu- and Nef-mediated CARDIF degradation to inhibit those host innate immune responses.



## Role of direct activation of B cells by CD40L in HIV virions in vivo in AIDS-NHL development

Marta Epeldegui

MIMG

B cell activation is frequently observed in HIV infected individuals. This immune activation may result from chronic antigenic stimulation and cytokine production. However, there is some evidence that HIV virions may directly activate B cells. We have previously observed that CD40L incorporated in the surface membrane of X4 and R5 HIV-1 has the ability to activate B cells in vitro. CD40L(+) HIV, but not CD40L(-), virions induced the expression of Activation Induced Cytidine Deaminase (AID) in vitro at the mRNA and protein level. Moreover, we have observed that HIV virions isolated from the plasma of HIV-positive individuals also contain virion-associated CD40L, and thus have the ability to induce B cell activation. In this study we are examining whether CD40L(+), but not CD40L(-), virions induce B cell activation in vivo using the Human Immune System (HIS) mouse model, which we have previously shown to be infectable with HIV-1. RAG2-/-c-/- mice were injected with human fetal liver CD34+ cells resulting in a successfully reconstituted human immune system. We created CD40L(+), CD40L(-) and CD40L(mut) virions by transfecting 293T cells (which naturally do not express CD40L) with an HIV-1 provirus lacking the envelope protein (pdENV), the envelope protein of an X4 HIV-1 (pHBX2) and CD40L (wildtype or mutant) or an empty control vector, thereby creating virions that can only infect T cells once. This is crucial in our experiments to infect with non-replicating virus so that the source of CD40L-bearing virions is only from the virions and not from the host T cells. Every two weeks for a total of 19 weeks, the HIS mice were injected with CD40L(+), CD40L(-) and CD40L(mut) non-replicative virions. At the same time the mice were bled to monitor their immune status and human B cell activation by multi-color flow cytometry. The following markers were examined: CD19, CD3, CD27, CD10, IgG, IgM, CD38, CD45RA, in addition to human CD45 and mouse CD45 to discriminate between human and mouse cells. The main immunophenotypic change we observed in the HIS mice after infection with CD40L(+) virions was an increase in CD10 expression. As early as 6 weeks after infection, CD10 was elevated in peripheral blood B cells of the mice infected with CD40L(+) virions as compared to mock, CD40L(-) and CD40L(mut) infected mice. Also CD10 expression was increased in splenic B cells of HIS mice infected with CD40L(+) virions, but not in mice that were mock infected or infected with CD40L(-) virions. Measurement of cytokine expression in plasma is ongoing. As CD10 is a marker of B cell activation and increased numbers of CD10+ B cells are seen in vivo in HIV infected subjects, the results suggest that CD40L incorporated in virions may have the ability to induce B cell activation in vivo.

# International Health Services and Policy Presentations

-  Jesse L. Clark, Infectious Diseases, “*Routine Screening for Acute and Recent HIV Infection in Lima, Peru*”
-  Risa Hoffman, Infectious Diseases, “*Impact of Antiretroviral Regimen and Duration on Risk of Mother-to-Child HIV Transmission in Johannesburg, South Africa*”

# Routine Screening for Acute and Recent HIV Infection in Lima, Peru

Jesse L. Clark

Infectious Diseases

**Background:** Screening for acute HIV infection is increasingly available in the United States and Western Europe, but rare in the resource-limited public health systems of developing countries. Prior to the implementation of routine screening programs for acute HIV infection in low- and middle-income countries, key issues including the cost, feasibility, and public health impact of different HIV testing strategies need to be determined. We evaluated the use of fourth-generation enzyme immunoassay (EIA) and pooled RNA PCR assays for the detection of acute and early HIV infection in routine counseling and testing settings in Lima, Peru.

**Methods:** Adults presenting for HIV testing at designated clinics were offered supplementary screening for acute HIV infection. All serum samples were tested with a fourth-generation Antigen/Antibody EIA (Vironostika Uni-Form II; Biomerieux). Positive results were confirmed by line immunoassay (LIA) (Inno-Lia HIV I/II Score; Innogenetics). All negative samples were combined into 50-sample master pools for HIV-1 RNA screening by PCR analysis (Taqman; Roche) according to a standard pooling algorithm. RNA-positive samples were re-tested with a third-generation EIA (Genetic Systems HIV-1/HIV-2 Plus O; Bio-Rad) to evaluate the relative sensitivity of standard testing procedures.

**Results:** Between December, 2007 and May, 2008 we recruited 1,191 participants. The prevalence of HIV infection in the entire sample was 3.2% (38/1191): 10.6% (25/237) among men who reported sex with men (MSM), 3.7% (4/109) among all other men, and 1.0% (8/821) among women. One case of acute HIV infection and one case of recent HIV infection were detected, both in participants identified as MSM. The prevalence of acute or recent HIV infection among participants defined as MSM was 0.8%. Fourth generation (p24) EIA detected one case of HIV infection not identified by third generation EIA. No additional cases of HIV infection were detected by pooled RNA screening compared with fourth generation EIA. Our findings are limited by the relatively small number of subjects in our pilot study and reflect the utility of screening for acute and recent HIV infection in Peru's concentrated HIV epidemic that may not be applicable to other resource-limited countries with generalized epidemics.

**Conclusions:** Our findings demonstrate the feasibility of screening for acute HIV infection within the constraints of Peru's resource-limited public health system and identify a high prevalence of acute and recent HIV infection among MSM in Lima. Additional studies are needed to fully evaluate the efficacy and cost of fourth generation EIA, RNA PCR, and other assays for the detection of acute and recent HIV infection when applied to larger-scale testing programs for high-risk individuals in Peru and other developing countries.

# Impact of Antiretroviral Regimen and Duration on Risk of Mother-to-Child HIV Transmission in Johannesburg, South Africa

**Risa Hoffman**

Infectious Diseases



**Background:** Limited information exists about effects of different highly active antiretroviral therapy (HAART) regimens and duration of regimens on mother-to-child transmission (MTCT) of HIV among women in Africa who start treatment for advanced immunosuppression.

**Methods:** Between January 2004 to August 2008, 1,142 women were followed at antenatal antiretroviral clinics in Johannesburg. Predictors of MTCT (positive infant HIV DNA PCR at 4-6 weeks) were assessed with univariate and multivariate logistic regression.

**Results:** Mean age was 30.2 years (SD=5.0) and mean baseline CD4 count was 161 cells/uL (SD=84.3). HAART duration at time of delivery was a mean 10.7 weeks (SD=7.4) for the 85% of women who initiated treatment during pregnancy and 93.4 weeks (SD=37.7) for those who became pregnant on HAART. Overall MTCT rate was 4.9% (43/874), with no differences detected between HAART regimens. MTCT rates were lower in women who became pregnant on HAART than those initiating HAART during pregnancy (0.7% versus 5.7%;  $p=0.01$ ). In a multivariate model that included baseline CD4 cell count and HAART regimen (NNRTI versus PI), duration of therapy was predictive of MTCT ( $p=0.02$ ). Each additional week of treatment reduced odds of transmission by 8% (95% CI: 0.87-0.99).

**Conclusions:** Our observational data highlights the importance of duration of HAART in women initiating therapy during pregnancy and demonstrates the high efficacy of long-term HAART in preventing MTCT in women becoming pregnant on therapy. Further efforts are needed to address social and health service barriers that may contribute to late identification of HIV-infected women in South Africa.

# Prevention and Clinical Research Presentations

-  John K. Williams, Psychiatry & Biobehavioral Sciences, “*Exploring Childhood Sexual Experiences and Vulnerability to Intimate Partner Violence among African American MSM/W*”
-  Chandra L. Ford, Public Health, “*An Evaluation of ‘Critical Race Theory and HIV/AIDS Disparities: A Multidisciplinary Think Tank*”

# Exploring Childhood Sexual Experiences and Vulnerability to Intimate Partner Violence among African American MSM/W

**John K. Williams**

Psychiatry & Biobehavioral Sciences

**Background:** While the HIV epidemic has disproportionately affected African American men who have sex with men (MSM), few HIV interventions have focused on African American men who have sex with both men and women (MSM/W). Childhood sexual abuse among MSM has been associated with increased sexual risk for HIV infection and poorer psychological outcomes, such as depression. For non-gay identifying (NGI) African American MSM/W, appraisal and self-definition of childhood sexual experiences may influence sexual identity and affect their ability to establish adaptive and safe physical and sexual boundaries. Additionally, these early sexual experiences may increase the risk of being in an adult relationship with intimate partner violence (IPV), specifically adult sexual abuse. Attention to the associations between appraisal of early sexual experiences and adult physical and sexual abuse need to be considered when developing HIV risk reduction interventions for HIV-positive NGI African American MSM/W.

**Methods:** Two groups of HIV-positive NGI African American MSM/W in Los Angeles, California participated in semi-structured focus group discussions, where each group met twice for 90-minutes. Thus, group A met twice for a total of 3 hours, as did group B. General discussions on childhood sexual experiences, appraisal and self-definition of these experiences, intimate adult relationships, and being HIV-positive in the African American community occurred at the initial meeting, with more in-depth exploration occurring at the second meeting. Eligible participants were HIV-positive African American men who had histories of sexual contact before the age of 18 years, had engaged in unprotected sex with both male and female partners in the prior 3 months and were non-gay identifying. Discussions were recorded, transcribed, and analyzed with a constant comparison qualitative method.

**Results:** Mean age of participants (n=16) was 40.5 years. The majority had a high school education (69%), with 33% earning an annual income of less \$10,000 and 50% reporting being “unable to work or unemployed.” While eligibility criteria required all participants to be NGI and behaviorally bisexual, on post-demographic survey 56% identified as gay and 13% as bisexual. Childhood sexual experiences were not perceived to be traumatic by 37%. Intimate partner violence, including both physical and sexual abuse, was viewed to be commonplace among African American heterosexual couples, but especially among male-male relationships by 56% (n=9). Reasons for physical and sexual violence included mirroring behaviors displayed by parents and violence being a proxy for manhood, strength, and love. Also reported was the occurrence of men being both victim and perpetrator in both male-female and male-male relationships. Only two participants acknowledged IPV as being a reason to terminate a relationship and only one acknowledged early sexual experiences as possibly contributing to unsafe adult sexual behaviors.

**Conclusions:** Understanding how NGI African American MSM/W interpret early sexual experiences may have an impact on sexual decision-making, sexual identity formation, and ability to form healthy adult sexual relationships. The impact of early sexual experiences, especially those that include negative appraisal and coercion must be considered when developing HIV risk reduction interventions for NGI African American MSM/W. Learning Objectives 1. To gain an understanding of the associations between early childhood sexual experiences and HIV sexual risk behaviors among non-gay identifying HIV-positive African American MSM/W. 2. To gain an understanding of the associations between early childhood sexual experiences and intimate partner violence among non-gay identifying HIV-positive African American MSM/W. 3. To gain an understanding of the associations between early childhood sexual experiences and sexual identity formation.

# An Evaluation of “Critical Race Theory and HIV/AIDS Disparities: A Multidisciplinary Think Tank”

**Chandra L. Ford**

Public Health

**Background:** In April 2009, the Department of Community Health Sciences in the UCLA School of Public Health with funding from the UCLA AIDS Institute convened “Critical Race Theory and HIV/AIDS Disparities: A Multidisciplinary Think Tank”, the first Critical Race Theory (CRT)-focused event nationally to be held within a public health context. The purpose of the one day Think Tank was to introduce CRT to the HIV prevention community and to promote its use in HIV/AIDS disparities research and practice. Although CRT has the potential to provide HIV/AIDS prevention researchers with useful tools for investigating and challenging racial and ethnic disparities, few researchers are familiar with the methodology. The Think Tank had four SPECIFIC AIMS: (1) To introduce the basic tenets of Critical Race Theory to HIV/AIDS researchers; (2) To explore key considerations for employing critical race methodologies in HIV/AIDS disparities research; (3) To generate an action plan for publishing research using the critical race public health methodology; and, (4) To begin to establish a network of researchers interested in advancing a critical race public health praxis. A clear understanding of the strengths and weaknesses of this first CRT gathering is necessary to advance the use of CRT approaches within the field of HIV prevention.



**Purpose** The purpose of this evaluation was to determine: (1) whether the Critical Race Theory and HIV/AIDS Disparities Multidisciplinary Think Tank achieved its aims as stated in the funding proposal; (2) the extent to which participants' perceived the event as helpful; and, (3) progress toward completion of proposed products.

**Methods:** The Think Tank aims and products (submission of 2 manuscripts) were evaluated using wording taken verbatim from the funding proposal. Participants' perceptions were assessed using a one-page evaluation. Participants completed and submitted the evaluation anonymously upon conclusion of the event. The evaluation assessed the helpfulness of materials, presentations and facilities; overall strengths and weakness of the event; and recommendations. Response options were on a Likert-type scale ranging from strongly disagree=1 to strongly agree=5. Analyses were completed using qualitative methods and simple frequency analyses.

**Results:** The Think Tank's four aims were achieved through its design and implementation; specifically, the educational format of the morning session; materials provided to participants; expertise of presenters; and, networking opportunities. Evaluations indicated very high levels of satisfaction (mean=4.83; range=4, 5). Key recommendations included hosting future CRT and public health events via webinar so that persons in other regions may participate. The product-related objectives were achieved. Three additional products not enumerated in the proposal also emerged from the event.

**Conclusions:** The “Critical Race Theory and HIV/AIDS Disparities: A Multidisciplinary Think Tank” achieved its stated objectives and was evaluated as very meaningful and important by participants. Products from the event include those enumerated in the proposal as well as others forged as a result of trans-disciplinary networking during the event. The overwhelming success of the gathering suggests strong support for future efforts to promote greater familiarity with Critical Race Theory among HIV/AIDS disparities researchers.

# Clinical Therapeutics and Biomedical Prevention Presentations

-  Andrew Levine, Neurology, “*Different Neurocognitive Outcomes in HIV+ Abstinent Cocaine versus Methamphetamine Users*”
-  Debika Bhattacharya, Infectious Diseases, “*Women Experience Higher Rates of Adverse Events During HCV Therapy in HIV Infection*”

# Different Neurocognitive Outcomes in HIV+ Abstinent Cocaine versus Methamphetamine Users

**Andrew Levine**

Neurology

**Background:** Both cocaine and methamphetamine use have been shown to result in neurocognitive deficits. Additionally, it has been shown that the adjunctive effect of HIV infection to cocaine or methamphetamine use results in even greater neurotoxicity, likely through oxidative stress or compromise of the blood-brain barrier, although the mechanism through which these two drugs exert these effects differs. This translates into subsequent neurocognitive deficits in HIV+ drug users that may be greater than the expected additive effects of HIV or drug use alone. However, to date no studies have examined the potentially different impact of cocaine versus methamphetamine use on neurocognitive functioning in those with HIV. In the current study, we compare HIV+ abstinent cocaine users to HIV+ methamphetamine users.

**Methods:** Patients were enrolled in the longitudinal National Neurological AIDS Bank (NNAB) study. Eighteen Caucasian HIV+ adults were included in the study. Nine met DSM-IV criteria for past cocaine abuse or dependence with no other past or current drug use disorder. Nine met similar criteria for past methamphetamine abuse or dependence, with no other past or current drug use disorder. All had reportedly been abstinent for at least one year. These patients had no history of non-HIV related neurological illness. The two groups were compared with regards to neurocognitive functioning via ANOVA. Groups were also compared on age, education level, and length of infection.

**Results:** The groups did not differ with regards to age, education, or length of infection. Both groups had an equal rate (33%) of HIV-associated neurocognitive disorders (HAND) at baseline visit. One individual in the methamphetamine group had current major depressive disorder. Cocaine users performed significantly poorer than methamphetamine users on the Digit Symbol-Coding test ( $p = 0.03$ ) and on the Symbol Search test ( $p=0.05$ ). Cocaine users also showed a trend toward poorer performance than methamphetamine users on the Paced Auditory Serial Addition Test (PASAT) ( $p=0.06$ ).

**Conclusions:** In our sample, despite similar demographic background and rate of HAND, cocaine users showed poorer performance than methamphetamine users on measures primarily assessing information processing speed. This finding is consistent with research that demonstrates a synergistic effect of HIV and cocaine. This synergism, in combination with a possibly limited recovery during abstinence, may result in an increased neurotoxicity in HIV+ abstinent cocaine users beyond that seen in HIV+ abstinent methamphetamine users.

# Women Experience Higher Rates of Adverse Events During HCV Therapy in HIV Infection

**Debika Bhattacharya**

Infectious Diseases

**Background:** In HIV/HCV coinfection, adverse events (AE) to HCV therapy account for 15% of treatment discontinuations. It is unknown whether sex influences complications.

**Methods:** Meta-analysis to study the effect of sex and other predictors of AEs in 3 randomized trials, A5071, APRICOT, and ANRSHC02-RIBAVIC that evaluated formulations of Interferon (IFN) and Pegylated IFN (PEG), both  $\pm$  Ribavirin in HIV/HCV coinfection. Primary endpoints were AEs requiring treatment discontinuation (AETD) or first dose modification (AEDM). Multiple stratified logistic regression was used to study predictors and assess interactions with sex.

**Results:** 21% of 1376 subjects were women; 61% had undetectable HIV RNA; 14% were antiretroviral therapy (ART) naïve at entry. Immune function was relatively preserved with median CD4 of 485 cells/mm<sup>3</sup>. Overall, 17% experienced an AETD and 50% AEDM; women had more AETD, 24% v 16% ( $p=0.003$ ) and AEDM (61% v 48%  $p<0.0001$ ). AETD and AEDM occurred earlier in women however the type of AETD and AEDM were similar between sexes. 74% of AETDs and 49% of AEDMs were constitutional (fatigue, headache). 18% of AETDs were depression while 26% of AEDM were neutropenia. Higher AETDs were seen with older age overall (OR=1.70 per 10yrs). We identified interactions with sex and BMI ( $p=0.04$ , continuous) and NNRTI ( $p=0.03$ ); More AETDs were observed in women on NNRTIs and in men with lower BMI. More AEDMs were seen with PEG (OR=2.07), increasing age (OR=1.48 per 10yrs), decreasing BMI (OR=1.04 per kg/m<sup>2</sup>), HCV genotype 1,4 (OR=1.31), Ishak 5,6 (OR=1.42), and decreasing Hgb (OR=1.23 per g/dL). Interactions between sex and ART-naïve status ( $p=0.001$ ) and AZT ( $p=0.001$ ) were identified; more AEDMs were in ART naïve women and prior treated men, and high AEDMs with AZT in women.

**Conclusions:** Although there was no difference in type of AE, women are more likely to have AETD or AEDM, and for them to occur more rapidly than in men. In women, antiretroviral regimen may be an important predictor of AETDs during HCV therapy and should be explored as a predictor of AEs in future HIV/HCV coinfection trials.

# Abstracts of Poster Presentations

- **Basic and Translational Research**

- ✚ Vaithilingaraja Arumugaswami, Mol. & Med. Pharmacology, "Developing a Cell Therapy Approach to Restrict HCV Infection and Promote Liver Regeneration"
- ✚ Marc Douaisi, MIMG, "Treg dysregulation in the thymus contributes to HIV-1 induced immune activation"
- ✚ Azadeh Farzin, Pediatric Infectious Diseases, "Role of Amniotic fluid and Vernix in In utero Transmission of HIV"
- ✚ Robert Furler, MIMG, "GLI Transcription Factors Induce TGF- $\beta$ 1 in Human CD4+ T-cells Following Activation"
- ✚ Lei Jin, MIMG/CNSI, "Structural studies of HIV envelop glycoproteins by cryoEM"
- ✚ Sanggu Kim, MIMG, "Analysis of long-term repopulating hematopoietic clones in a rhesus macaque by a novel clonal tracking assay"
- ✚ Martha J. Lewis, Infectious Diseases, "In vitro immune selection reveals HIV-1 NEF sequence motifs important for its immune evasion function in vivo"
- ✚ Roshni Mehta, Hematology/Oncology, "Activation of latent HIV-1: A means of purging viral reservoirs"
- ✚ Koki Morizono, Medicine, "Redirecting HIV vectors to DC-SIGN by modification of N-linked glycans of envelope proteins"
- ✚ Angela Presson, Public Health -Biostatistics, "Statistical methods to detect viral integration site hotspots"
- ✚ Dimitrios N. Vatakis, Hematology/Oncology, "Understanding the block to HIV infection in quiescent CD4+ T cells"
- ✚ Jun Zuo, Pediatrics, "Enhanced CD4 T cell differentiation associates with CD4 T cell loss in HIV-positive adolescents"

- **Clinical Therapeutics and Biomedical Prevention**

- ✚ Andrew Levine, Neurology, "A candidate gene study of HIV-dementia in the National NeuroAIDS Tissue Consortium"

- **International Health Services and Policy**

- ✚ Angela Bayer, Infectious Diseases, "Perceived HIV-related Risks among Young People in Peru"

- **Prevention and Clinical Research**

- ✚ Bitu Amani, Public Affairs, "Let's Get Physical: Sexually Transmitted Infections and Body Mass Index among US Young Adults"
- ✚ Ryan Murphy, Epidemiology, "Differences in Sexual Position Between Men Who Have Sex with Men (MSM) and Men Who Have Sex with Men and Women (MSMW)"
- ✚ Allison Ober, Social Welfare, "The Relative Role of Perceptions of Partner Risk in Unprotected Sex"
- ✚ Nava Yeganeh, Pediatric Infectious Disease, "Feasibility of voluntary testing and counseling for the partners of pregnant woman in prenatal care"
- ✚ Sean Young, Medicine, "Seeking Psychological Cover: The Relationship between HIV Testing, Health Services Usage, and Stigma"

# Developing a Cell Therapy Approach to Restrict HCV Infection and Promote Liver Regeneration

Vaithilingaraja Arumugaswami

Mol. & Med. Pharmacology

Hepatitis C Virus (HCV) is a major human health concern. Globally, an estimated 170 million people are infected with HCV. HCV causes liver diseases including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Currently no vaccine is available and the available treatments have limited response rate. Liver transplantation is the only treatment option for advanced liver diseases. Unfortunately, circulating HCV readily infects the newly transplanted healthy liver and leads to its dysfunction. Repopulating the decompensated liver with autologous hepatocytes rendered resistant to hepatitis C viral infection is a promising therapeutic option. Depleting or silencing cellular and viral factors critical for HCV replication can limit HCV infection. Targeting the conserved sequences of the HCV genome through RNA interference has inhibited HCV replication. HCV utilizes host machinery for its propagation. For entry, HCV uses a number of cell surface receptors such as CD81 (major receptor type), Scavenger Receptor B-1, Claudin-1, Claudin-6, Claudin-9, and Occludin. Currently, we are in the process of testing and identifying efficient RNAi target sequences against these cellular HCV receptors. The shRNA targeting cellular and viral factors will be delivered into human primary hepatocytes or patient specific induced pluripotent stem (iPS) cell derived-hepatocytes through lentiviral vectors. We have observed that fetal hepatocytes can be infected by the HCV strain J6/JFH-1. We were able to generate hepatocyte-like cells from human embryonic stem cells. Silencing cellular factors can lead to the development of diseases associated with genetic defects in an individual. Currently, the side effects of knocking down HCV entry receptors at the tissue or organismal level are unknown. In order to understand the phenotype resulting from silencing HCV entry receptors, initially we will assess the ability of the genetically altered hepatocytes to form functional liver tissue and liver architecture in a humanized FRG mouse model. This approach will be further developed for patient specific liver regenerative medicine.

# Treg dysregulation in the thymus contributes to HIV-1 induced immune activation

Marc Douaisi

MIMG

Regulatory T cells (Treg) develop in the thymus and are critical for the control of immune responses. Treg may be beneficial by limiting general non-specific immune activation, a hallmark of HIV-1 infection. However, Treg may also be detrimental by suppressing HIV-specific immune responses thus allowing more virus replication. The impact of HIV-1 on the development of FoxP3<sup>+</sup> T cells (Treg) in the thymus remains unclear. The goal of our research is to analyze the mechanisms by which HIV-1 affects Treg numbers and function after infection with X4-HIV-1 and R5-HIV-1. Using the conventional SCID-hu thy/liv mouse model, we compared the impact of X4-HIV-1 (NL4-3) vs. R5-HIV-1 (JR-CSF) infection on FoxP3<sup>+</sup> Treg in the thymus. Thymus/liver (thy/liv) grafts in SCID-hu mice were infected with HIV-1 and thymocytes were obtained for immunophenotypic analysis at various time points post-infection. We observed an increase in the percentages of FoxP3<sup>+</sup> thymocytes after infection with both X4- and R5-HIV-1. However, the increase in FoxP3<sup>+</sup> thymocytes occurred much faster with the more cytopathic isolate NL4-3 (X4) than with R5-HIV-1. Moreover, we found a differential effect of X4- and R5-HIV-1 on FoxP3<sup>+</sup> thymocytes. Specifically, we showed that, in CCR5 (R5)-tropic JR-CSF infected tissues, FoxP3<sup>+</sup> thymocytes are up to 3 fold more productively infected (HIV<sup>+</sup>) than FoxP3<sup>-</sup> mature thymocytes. In contrast in CXCR4 (X4)-tropic NL4-3 infected tissues, FoxP3<sup>+</sup> cells express up to 4 fold less HIV-1 Gag than their FoxP3<sup>-</sup> counterparts. Thus, our results show that a higher percentage of Treg is productively infected by R5-HIV-1 than by X4-HIV-1 in the human thymus. As productively infected cells are very likely to die, we hypothesize that R5-virus decreases the proportion of Treg in the thymic output, whereas X4-HIV-1 causes an increase in Treg in the thymic output. Using our novel “humanized” mouse model, bearing a human immune system, we will determine whether the increase in Treg in the thymus causes an increase of Treg in the blood and peripheral organs of the immune system (spleen, lymph nodes). Such a modification of the thymic output can lead to perturbation of HIV-specific immune responses in the periphery and/or interfere with the control of autoimmunity. It should also be noted that during the natural course of HIV-1 infection, R5-HIV-1 is initially the dominant type of virus while the switch to X4-strains occurs later during the asymptomatic phase and is associated with disease progression to AIDS. An increase in peripheral Treg after X4-HIV-1 infection of the thymus will result in an impaired immune response to the virus, thereby contributing to the progression to the symptomatic phase of HIV infection after tropism switch from R5-HIV-1 to X4-HIV-1.

# Role of Amniotic fluid and Vernix in In utero Transmission of HIV

**Azadeh Farzin**

Pediatric Infectious Diseases

Pediatric HIV infection is a global public health challenge with an estimated 1,200 children newly-infected every day. Intrauterine HIV transmission accounts for 25% to 40% of total cases of perinatally acquired HIV among non-breastfed infants with an absolute risk of 5-7% of births among non-treated mother-infant pairs. Understanding events that transpire in utero which result in HIV infection of the baby, as well as immune mechanisms that reduce infectivity of the fetus, may ultimately lead to interventions that can reduce in utero transmission of HIV and potentially other intrauterine infections. Indirect evidence—further supported by preliminary data from our laboratory—on in utero transmission of HIV suggests a role for amniotic fluid (AF) in absorption of the virus through the fetus' immature mucosal surfaces such as the digestive tract. If the fetus is indeed exposed to HIV via AF, it seems likely that in utero transmission rates would be higher than currently observed, and therefore natural protective mechanisms may exist to prevent HIV-1 transmission in utero. During gestation and soon after birth, all infants rely upon protective effects of vernix and AF. Production of vernix, a cheesy substance composed of proteolipids and synthesized by fetal sebaceous glands, begins during the third trimester in all infants and often covers the full term infant's body at the time of birth. In utero, vernix is dissolved by pulmonary surfactant into the AF and is swallowed by the developing fetus, introducing defensive substances into the digestive tract. Current literature supports a strong role for antimicrobial peptides of the innate immune system in protection of the fetus. Some of the antimicrobial peptides recently identified in amniotic fluid and vernix have been shown to exhibit anti-HIV activity. The role of immune factors in amniotic fluid and vernix in protection of the fetus against HIV has not been investigated.

During this two year project, we will investigate the following three specific aims: A) To identify and quantitate critical antimicrobial peptides in AF and vernix which may contribute to reduced intrauterine HIV infection of the fetus. B) To test the hypothesis that presence of HIV-1 in amniotic fluid plays a role in in utero transmission of the virus. C) To delineate the potential route of intrauterine HIV-1 transmission via phylogenetic study of HIV envelope glycoprotein (Env) genome, hence determining the temporal relationship between maternal, intrauterine and infant HIV strains. During this prospective pilot study, specimens including amniotic fluid, vernix and potentially placenta, maternal and infant blood will be collected in three groups of pregnant women and infants: Group 1) Control population to establish baseline levels of AMP among HIV negative mothers and infants; Group 2) Mother-baby pairs with maternal HIV, but without in utero transmission of HIV; Group 3) Mother-baby pairs with maternal HIV and in utero transmission of HIV.

Our current progress includes sample collection in twenty control mother-infant pairs. We are currently awaiting IRB approval in southern Brazil, where we plan to enroll majority of our HIV positive patient population. Our preliminary data from in vitro studies on specimen from control subjects suggests an inhibitory role by AF on HIV infectivity of normal Peripheral Blood Mononuclear Cells, supporting our hypothesis. Our immediate next steps include confirming the preliminary data, performing a similar experiment on vernix and potentially fractionating AF and vernix to identify antimicrobial peptides or proteins with anti-HIV activity. Specimens collected from HIV positive patients will be analyzed for the 1) Presence of HIV in AF and vernix, as a potential route of in utero transmission and 2) Analysis similar to control subjects to confirm in vivo role of antimicrobial peptides.

# GLI Transcription Factors Induce TGF- $\beta$ 1 in Human CD4+ T-cells Following Activation.

Robert Furler

MIMG

**Background:** The depletion of Th17 and increase of Treg in the gut have been implicated in chronic immune activation in HIV-infected patients and NHP models of progressive HIV infection. The balance of human Th17 and Treg cells can be altered by TGF- $\beta$ 1, a pleiotropic cytokine that has been shown to be induced by HIV-1 Tat and is increased in progressive HIV-1 disease. We have investigated the role of the human GLI proteins, which have previously been reported to interact with Tat and transactivate the LTR of HIV-1, in TGF- $\beta$ 1 induction. Here we report that GLI proteins regulate TGF- $\beta$ 1 during CD4+ T-cell activation which may lead to the skewing of human CD4+ T-cells from Th17 cells, that protect mucosal barrier integrity, to iTreg that can suppress HIV-specific immune responses.

**Methods:** Human naïve CD4+ T-cells were negatively selected from PBMC and stimulated with aCD3/aCD28 microbeads for 24 hours prior to RNA extraction. RT-PCR of GLI1 mRNA was done and standardized to 18S rRNA to test the activation state of GLI2 and GLI3 following CD4+ T-cell stimulation. For in silico screening of putative GLI binding sites in the human TGF- $\beta$ 1 promoter 293T cells were cotransfected with TGF- $\beta$ 1 promoter-luciferase constructs along with pGli2.N (activator), pGli3PHS (repressor), or pcDNA3 control. Putative GLI-binding sites were mutated using site-directed mutagenesis. Luciferase activity was read 48h post-transfection. The Wilcoxon Rank Sum test was used for statistical analysis of the data.

**Results:** We found that there are six putative GLI-binding sites surrounding the human TGF- $\beta$ 1 promoter. RT-PCR of GLI1 mRNA, a marker for GLI2 and GLI3 activation, confirmed that stimulation of CD4+ T-cells through CD3/CD28 activates GLI2 and GLI3 which induces GLI1 mRNA >10-fold and TGF- $\beta$ 1 mRNA 4-fold. A constitutive Gli activator (GLI2.N) significantly induced transcription at the TGF- $\beta$ 1 promoter by 4-fold over control. Mutagenesis of the putative GLI binding sites abrogated this induction. Subsequently, a constitutive GLI repressor (GLI3-PHS) significantly repressed TGF- $\beta$ 1 transcription 14-fold over control.

**Conclusions:** The GLI proteins which have previously been shown to transactivate the HIV-1 LTR, are activated following CD4+ T-cell stimulation and induce TGF- $\beta$ 1 by binding to its promoter. The increased levels of TGF- $\beta$ 1 induced by the GLI proteins may be one of the underlying factors for the Th17/Treg imbalance seen in HIV-1 disease.

# Structural studies of HIV envelop glycoproteins by cryoEM

Lei Jin

MIMG/CNSI

**Background** The surface glycoproteins of enveloped human immunodeficiency viruses (HIV) mediate cell attachment and membrane fusion, thus allowing the virus to enter a new host cell. As the major surface antigen, the envelop glycoproteins also play a key role in the virus's ability to evade the immune system. The glycoprotein forms trimeric spikes on the surface of mature virion, where the receptor-binding subunit gp120 and the fusion subunit gp41 are non-covalently associated. Extensive structural work has been done with monomeric gp120, but the conformational organization and the conformational rearrangements associated with CD4 binding in a trimer context are still elusive. Attempts to crystallize these trimers for X-ray structure determination have so far unsuccessful, despite many years of effort. This has hindered structural studies on HIV envelop glycoproteins by X-ray crystallography. Recently, cryo-electron tomography (cryoET) has been exploited to reveal the glycoprotein structure, but the resolution is still too low ( $\sim 30\text{-}40\text{\AA}$ ) to reveal any detailed conformations and structural changes for cell attachment and entry. The long-sought-after and much-needed high-resolution structural information about the trimeric envelope glycoproteins is vital in HIV vaccine development and new anti-viral therapy design.

**Goal and approach:** To fill the missing knowledge gap, we are performing structural studies on the HIV trimeric envelop glycoproteins by a new emerging technology: high-resolution single-particle cryo-electron microscopy (cryoEM). CryoEM is a set of techniques for solving structures of biological specimens by processing images of an object taken by transmission electron microscopy at cryogenic temperature. The advantage of CryoEM is that the sample does not have to be in a crystal form and can be directly imaged in their solution conformation. As a first attempt, we have chosen HIV gp140 as a target. The goal is to get  $\sim 10\text{\AA}$ -resolution density map for gp140 to establish the feasibility of this cryoEM approach.

**Progress:** HIV gp140 has been purified in its trimeric form by size-exclusion chromatography. We have seen distinct gp140 practices under negative-staining electron microscope, setting up a basis for the next step of cryoEM studies. Preliminary cryoEM imaging showed promising power spectrum for the particles. Further optimization of gp140 sample such as concentration and freezing conditions for cryoEM imaging is under way.

# Analysis of long-term repopulating hematopoietic clones in a rhesus macaque by a novel clonal tracking assay

Sanggu Kim

MIMG

As a potential gene therapy for AIDS, we have demonstrated stable reduction of CCR5 by RNAi in non-human primates through lentivector-mediated, hematopoietic stem/progenitor cell (HSPC) transplant. Gene therapies utilizing stem cells and retrovirus vectors suffer from insufficient control over the growth of individual stem cells harboring a therapeutic vector inserted at different locations in the genome. The risk of premature implementation of clinical trial has been poignantly demonstrated by the subsequent development of leukemia in four children in the onco-retrovirus (MLV) vector-mediated gene therapy for X-linked severe combined immunodeficiency. To date, however, lentiviral vector transduction of HSPC has not shown evidence of malignant transformations due to insertional mutagenesis. For the realization of stem cell gene-therapies in clinic, it is important to clearly understand the biology of individual stem cells following transplantation. We report here the first in-depth study of long-term repopulating HSPC clones in a rhesus macaque stably repopulated in multiple hematopoietic lineages for up to 10 years following transplant with autologous CD34+ cells transduced with self-inactivating lentiviral vectors. We used a novel clonal tracking assay which we term Pyrosequencing-based Quantitative Clonal Tracking (PQCT) for sensitive, high-throughput quantitation of repopulating HSPC clones in vivo. The assay involves large-scale sequencing for vector integration sites (VIS) as markers of individual clones to simultaneously assay both the genomic location as well as sequence frequencies of thousands of individual VIS. We analyzed 1330 VIS in repopulating peripheral blood mononuclear cells (PBMC) at 9 time points over 10 years. The frequencies of individual VIS were highly heterogenic ranging from <0.01% to 4% of total vector copy and showed an evolution from polyclonality at early time points following transplant to oligoclonality at later time points. Furthermore, we observed the VIS in repopulating cells are clustered into a number of genomic loci (hotspots) compared to those in acute infection. We quantified the regions of such hotspots by a novel Bayesian change point framework to determine the location of hotspots from consecutive changes in integration rate along a chromosome. In repopulating PBMC, nearly half of the VIS are located in 78 hotspots covering about 1.6% of the genome with an average size of 647kb per hotspot. Gene profile analysis indicates that hotspots are enriched for genes that are significantly upregulated in PBMC compared to CD34+ cells and are associated with immune function or cellular development. These data demonstrate detailed kinetics of long-term repopulating HSPC clones and the association of VIS with engraftment and repopulation of HSPCs.

# In vitro immune selection reveals HIV-1 NEF sequence motifs important for its immune evasion function in vivo

**Martha J. Lewis**

Infectious Diseases

**Background:** The HIV Nef protein plays a key role in pathogenesis through the downregulation of major histocompatibility complex (MHC) class I proteins. This downregulation has been shown to impair the recognition and killing of infected cells by cytotoxic T lymphocytes (CTLs) thus contributing to persistent infection. Important sequence motifs associated with Nef's function have largely been determined by mutational analysis of laboratory strains. It is not known if the same sites are important for the function of primary isolates. Previously, we found that downregulation of MHC-I by the circulating Nef quasispecies in vivo often yields a bimodal distribution of populations that either fully downregulate (compared to NL4-3 Nef) or do not downregulate. We therefore subjected primary Nef quasispecies to in vitro immune selection with an HIV-1-specific CTL clone, and subsequently examined the selected isolates for sequence and functional evolution.

**Methods:** Nef quasispecies were amplified from plasma of 9 chronically infected individuals and cloned into an NL4-3 based vector. Recombinant viruses were used to infect T1 cells. Infected cells were cultured in parallel in the presence or absence of an HIV-specific CTL clone. Multiple full-length nef clones were sequenced for each subject to create 3 sequence data sets – one with the input sequences, one after CTL selection, and one without CTL selection. The multiple measures of adaptive evolution were compared between the datasets using nucleotide and protein sequence analysis to identify specific polymorphisms and patterns of evolution associated with resistance to CTL killing. Finally, Nef's MHCI downregulation function was measured for each subject before and after selection.

**Results:** 259 sequences were analyzed (100 input, 78 with CTL, 81 without CTL). At baseline, fewer than 5% of all sequences contained mis-sense mutations. Among selected sequences, previously identified functional domains (e.g. EEEE and PxxP motifs) were either intact or contained known polymorphisms, and there were no consistent substitutions associated with selection. Culture with CTL was associated with decreased non-sense mutation and diversity and a statistically significant lower global dN/dS ratio. 13 of 206 sites had evidence of purifying selection and 1 site had evidence of positive selection. 7 of 13 negatively selected sites had previously been associated with Nef function. At baseline some subjects had only partial function to downregulate MHCI, but the changes in sequences with CTL selection were accompanied by a recovery of function.

**Conclusions:** The nef quasispecies under CTL selection display a pattern of strong purifying selection associated with optimization of its MHCI downregulation function. These results demonstrate strong evolutionary pressures on Nef via its functional role in immune evasion, while the lack of consistently selected polymorphisms indicate the plasticity of this small protein to adapt to maintain function through diverse genetic pathways.

# Activation of latent HIV-1: A means of purging viral reservoirs

**Roshni Mehta**

Hematology/Oncology

HIV-1 infected patients undergoing highly active antiretroviral treatment (HAART) maintain undetectable virus loads (<50 copies of virion RNA/ml) for extended periods of time. However, upon stopping HAART the virus re-emerges very rapidly and can be found in the plasma. These same patients on HAART have been shown to harbor a subset of latently infected CD4<sup>+</sup> T-lymphocytes and it is believed that these cells could account for the viral rebound after stopping HAART. Until therapies are developed to target these latently-infected cells, HIV cannot be cleared from the body.

Our lab has previously developed an animal model to study HIV latency in primary human thymocytes. This model has been used to generate large numbers of latently infected human cells, perform small scale screening for activators of latent HIV and also to identify signaling pathways involved in the stimulation of HIV in primary cells. Although this model has been used to gain understanding of latent HIV in primary cells, it is limited by expense, technical complexity and a longer experimental turn around time than most non-animal (in vitro) models.

Therefore we have also generated an in vitro model of latent HIV in primary human thymocytes. Activated primary thymocytes are infected in vitro with a reporter virus that expresses green fluorescent protein (GFP) and luciferase under control of the HIV LTR (promoter). The thymocytes differentiate after 7-10 days in culture and become quiescent but can be activated (stimulated) by addition of CD3/CD28 antibodies which mimics T-cell activation. This model is capable of generating large numbers of latently infected thymocytes that can induce viral expression >200 fold when stimulated as measured by luciferase production. We now aim to adapt this model to perform high-throughput screening (HTS) of compounds to identify those which activate latent HIV. In this way we aim to gain a more complete understanding of the regulation of HIV latency in primary cells and ultimately be able to eradicate HIV from infected individuals.

# Redirecting HIV vectors to DC-SIGN by modification of N-linked glycans of envelope proteins

**Koki Morizono**

Medicine

HIV vectors have become very powerful tools in biology and gene therapy because of long-term expression of their transgenes and their ability to transduce non-dividing cells. Most of the current HIV vectors are pseudotyped with the VSV-G envelope protein, which confers high infectivity for a wide variety of cell types. Although effective for in vitro and ex vivo transduction, VSV-G-pseudotyped HIV vectors will transduce many tissues and organs non-specifically when administered in vivo, which reduces the therapeutic effects of transgenes in target tissues and increases their adverse effects. The best gene therapy vectors for in vivo administration are those that can specifically home in on target cells and tissues after intravenous administration, known as “targeting vectors”. We have been developing targeting HIV vectors by pseudotyping the vectors with modified Sindbis virus envelope proteins. The Sindbis virus envelope protein consists of two glycoproteins, E1 and E2. E1 mediates fusion between the target cell membrane and the viral envelope and E2 mediates binding of virus to cells. We mutated receptor-binding regions of the E2 protein to eliminate the original tropism of the envelope proteins, which prevented transduction of cells. When the E2 protein is conjugated with targeting ligands either covalently or non-covalently, the pseudotyped HIV vectors specifically transduce cells expressing receptors recognized by the conjugated ligands. For example, when integrin-targeting peptides were inserted into E2 proteins, the pseudotyped HIV vectors infected cells via the interaction of the inserted peptides and integrins expressed on target cells. When the E2 protein was conjugated with monoclonal antibodies recognizing tumor antigens, the HIV vectors specifically transduced tumor cells both in vitro and vivo.

We also attempted to redirect the pseudotyped vectors by modification of the structure of N-linked glycans. Sindbis virus grown in mosquito cells can infect cells, using DC-SIGN, a C-type lectin binding to high-mannose glycans, as its receptor; hence, the virus grown in mammalian cells cannot. This difference arises from the variance in the structures of N-glycans between the viruses derived from mosquito (all structures are high-mannose) and mammalian cells (mixture of high-mannose and complex). Since HIV vectors are produced in mammalian cells (human 293T cells), HIV vectors pseudotyped with modified and wild-type Sindbis virus envelope proteins could not use DC-SIGN as their receptor. To change all of the structures of N-glycans of the vectors to high mannose, we produced HIV vectors pseudotyped with modified Sindbis virus envelope protein (2.2 1L1L) in the presence of the inhibitor of complex N-glycan synthesis, 1-deoxymannojirimycin (DMNJ). The virus was designated DMNJ-2.2 1L1L. It specifically transduced cells expressing DC-SIGN. This transduction is blocked by antibodies against DC-SIGN and the soluble sugar, mannan, which demonstrates that transduction is mediated by DC-SIGN. We also attempted to identify the N-glycans of envelope proteins by mutating amino acids (asparagines) that links glycans. We found that N-glycans that were exposed on the surface of the envelope proteins (E1-139 and E2-196) mediate binding to DC-SIGN. Because DC-SIGN is highly expressed on antigen-presenting cells, such as dendritic cells and macrophages, our DC-SIGN-targeting vectors will be efficient tools to specifically deliver transgenes into these cells, which can be applied to genetic immunization.

# Statistical methods to detect viral integration site hotspots

**Angela Presson**

Public Health -Biostatistics

Modern gene therapy methods suffer from insufficient control over where a therapeutic viral vector inserts into the host genome. Viral integration patterns vary by vector type and often prefer regions containing genes, so that the probability of an integration event varies by location. Since integration can activate local gene expression, it is important to characterize insertion patterns of potential gene therapy vectors. Currently, viral integration hotspots are defined by a minimum density of events (2-4 within a 36-104kb region). While this guideline may be useful for some data sets, it relies on the number of observed integration sites. This is problematic for comparing hotspots among different vectors or collections of experiments where the number of observed integration sites can vary substantially. Furthermore, this definition targets small genomic regions. As the definition of viral integration hotspots is essential to understanding their mechanism and safety, an accurate and more general definition is warranted. We propose a Bayesian change point (bcp) framework to estimate integration hotspots from consecutive changes in integration rate along a chromosome. We identify change points using both a) a traditional bcp model and b) a modified version that samples the number of change points as a proposal in the Markov chain. We test these models on 1) our data from rhesus macaque animals transplanted with hematopoietic stem cells containing lentivirus vectors (LV), 2) published LV data, 3) published retrovirus data and 4) control data from pre-transplant stem cells containing LV. Preliminary results identified 35 hotspots in our LV transplant data (1). While the LV control data (4) contained four times the number of integration sites observed in (1), the bcp method identified only two additional hotspots. In comparison, a definition of  $\geq 2$  insertions within a 100kb region identified 43 hotspots in (1) and 150 in (4). The published transplant data sets (2-3) also suggest preferential integration. The average hotspot size in our LV data was about 850kb. Bayesian change point models effectively distinguish true hotspots from random integration events. Statistical models that can reliably define hotspots will allow us to compare integration preferences among different vector types and assess their safety and efficacy for gene therapy trials.

# Understanding the block to HIV infection in quiescent CD4<sup>+</sup> T cells

**Dimitrios N. Vatakis**

Hematology/Oncology

Quiescent CD4<sup>+</sup> T cells have been shown to be resistant to human immunodeficiency virus (HIV) infection. Earlier studies by our group demonstrated that quiescent CD4<sup>+</sup> T cells were unable to support productive viral replication characterized by incomplete reverse transcription. However, the permissiveness of other non-dividing cell types such as macrophages raised further questions regarding the nature of the block. Later studies further elucidated which subsets of resting cells were refractory to infection. Cells in the Go/1a phase, truly quiescent, were resistant to infection while cells in the G1b phase, characterized by high levels of RNA synthesis but not DNA, were susceptible to infection. Treatment of quiescent cells with nucleosides did not lead to a productive infection suggesting that other factors may contribute to resistance to infection. Recent studies examined the presence or absence of cellular factors responsible for the block. Both Murr1 and APOBEC3G have been shown to influence viral life cycle in quiescent CD4<sup>+</sup> T cells; however, they are not the sole inhibitory factors. To further examine HIV infection in quiescent CD4<sup>+</sup> T cells, we undertook a more comprehensive study and examined multiple stages of viral replication (entry, reverse transcription, integration, viral gene transcription and viral protein synthesis) in quiescent cells and compared to stimulated cells. Quiescent CD4<sup>+</sup> T cells were infected and then immediately stimulated to determine if this will rescue infection. Replication in these cells was characterized by a large delay in reverse transcription and integration when compared to pre-stimulated T cells. Reverse transcription was largely inefficient (30-fold less than stimulated cells), integration efficiency was slightly decreased (2-fold) and protein expression was very poor. Thus, immediate stimulation failed to effectively rescue infection of quiescent cells. Interestingly, their kinetics of infection were very comparable to that of infected quiescent cells with latter cell type not expressing any protein. These data suggested that the major block of HIV infection in quiescent T cells might occur before or at the early stages of reverse transcription and that the stages of the viral life cycle are defective. The presence of provirus in quiescent CD4<sup>+</sup> T cells that does not or poorly produce virions prompted us to look at the patterns of integration in quiescent CD4<sup>+</sup> T cells and comparing against their stimulated counterparts. The lack of protein expression in latently infected quiescent T cells suggests that the integration sites may be distinct in this cell subset. T cell quiescence is not a default but rather an actively maintained state regulated by lung Krüppel-like factor (LKLf) and Tob. Thus, it would seem natural based on the patterns of HIV integration that genes regulated by these two factors will be the preferred sites of integration. To address the above, we isolated quiescent CD4<sup>+</sup> T cells and along with stimulated cells we infected them with the CXCR4-tropic HIV-1NL4-3. Samples were harvested and HIV integration sites were sequenced and mapped. Based on our results, the chromosomal features associated with the entire integration between the two cell types were similar but the process was less efficient in quiescent cells characterized by a high frequency of abortive LTR-circles.□

## Enhanced CD4 T cell differentiation associates with CD4 T cell loss in HIV-positive adolescents

Jun Zuo

Pediatrics

Immune hyperactivation and increased turnover of CD4<sup>+</sup> T cells have been proposed as underlying mechanisms of CD4<sup>+</sup> T cell depletion during the progression to AIDS. Suppressive antiretroviral therapy often results in restoration of CD4<sup>+</sup> T cells in HIV-positive individuals but pathogenic mechanisms may continue to erode CD4<sup>+</sup> T cell population. In present study, we examined CD4<sup>+</sup> T cell proliferation using Ki67 staining in HIV-positive adolescents/young adults who perinatally acquired HIV and were receiving antiretroviral therapy. Percentages of Ki67<sup>+</sup> cells in naïve CD45RA<sup>+</sup> CD28<sup>+</sup> CD4<sup>+</sup> T cell subsets were significantly higher in HIV-positive subjects than in HIV-negative controls, and the increase of Ki67<sup>+</sup> cells persisted even in subjects whose HIV replication was under control (< 400 copies/ml for over a year). The results also showed that Ki67<sup>+</sup> cells in CD45RA<sup>+</sup> CD28<sup>+</sup> naïve CD4<sup>+</sup> T cell subpopulation were CD95<sup>+</sup> Hi CCR5<sup>+</sup>, suggesting differentiation into memory-like T cells. Furthermore, percentages of Ki67<sup>+</sup> cells in memory CD45RA<sup>-</sup> CD28<sup>+</sup> CD4<sup>+</sup> T cells correlated with plasma HIV levels. These Ki67<sup>+</sup> cells in memory CD4<sup>+</sup> T cell subpopulation downregulated the chemokine receptor CCR7 but upregulated CCR5, showing differentiation from central-memory T cells to effector-memory T cells. Indeed, higher percentages of Ki67<sup>+</sup> cells in memory CD4<sup>+</sup> T cell subpopulation were linked to lower levels of central-memory T cells and higher levels of effector-memory T cells in HIV-positive subjects. Finally, we found that total CD4<sup>+</sup> T cell levels in peripheral blood positively correlated with percentages of central-memory CD4<sup>+</sup> T cells and negatively with percentages of effector-memory CD4<sup>+</sup> T cells. We concluded that enhanced cell differentiation persisted during chronic HIV infection even with successful viral suppression and that central-memory to effector-memory differentiation was especially important process underlying CD4<sup>+</sup> T cell decline amid progression to AIDS.

# A candidate gene study of HIV-dementia in the National NeuroAIDS Tissue Consortium

**Andrew Levine**

Neurology

**Background:** HIV-associated dementia continues to occur with a significant prevalence despite widespread use of antiretroviral regimens and longer survival time. Already, a number of gene variants have been identified that alter HIV disease progression and risk for HAD. However, findings have not commonly been replicated and most studies failed to consider the multitude of environmental and lifestyle factors that might themselves confer risk for HAD. In the current study, we sought to replicate the findings of previous studies in a neurologically and behaviorally well-characterized cohort.

**Methods:** 143 HIV+ individuals enrolled in the National NeuroAIDS Tissue Consortium (NNTC) were included in the analysis. Based on consensus diagnosis, 117 were considered neurologically normal upon study entry, and 26 had HAD. Seven single nucleotide polymorphisms (SNPs) were genotyped within 7 genes (CCL2, CCL3, CCL5, IL-1a, IL-10, SDF-1, and TNF-alpha). Logistic regression analysis was used to predict group membership (normal vs. HAD), with predictor variables including length of infection in years, age, current stimulant dependence, current depression, and genotype.

**Results:** The two groups were statistically similar with regards to demographic characteristics, drug use history, and disease factors. The HAD group had significantly greater number of individuals with current depression. Only one SNP, rs1130371 within the gene for CCL3, was entered into the analysis as the others showed symmetric distribution between groups. Logistic regression indicated that depression and CCL3 genotype were significant predictors of HAD. Depression conferred a 5-fold greater risk of HAD, while the TT genotype for CCL3 SNP (rs1130371) was associated with two-fold risk for HAD. No other gene was found to predict HAD in our sample.

**Conclusions:** This study found that depression and CCL3 genotype predicted HAD in an ethnically mixed HIV+ sample. The fact that SNPs previously found to be associate with HAD were not in our analysis, and that rs1130371 is in high linkage disequilibrium with neighboring genes indicates that more dense genotyping in significantly larger cohorts is required to understand the true relationship between genotype and risk for HAD.

# Perceived HIV-related Risks among Young People in Peru

**Angela Bayer**

Infectious Diseases

**Background:** There are currently 5.3 million 15-24 year olds in Peru, all of whom represent an enormous resource for the country's development. Youth, however, confront numerous challenges, including HIV. Youth vulnerability to HIV is multi-dimensional: young people experience numerous transitions as adolescents and youth; young people face diverse vulnerabilities in their daily lives; and HIV is a risk that is ever-present for young people. More than half of the overall HIV/AIDS cases reported in Peru have been in 15-34 year olds. Therefore, the objective of this study is to explore what the Peruvian youth think about their HIV-related risks and vulnerabilities.

**Methodology:** Focus group discussions and in-depth interviews were carried out with 141 Peruvian 18-24 year olds from January-March, 2009. The following groups of participants were recruited in collaboration with local organizations from Lima/Callao (capital/coast), Arequipa (highlands) and Iquitos (jungle): heterosexual males and females, gay males, transgendered individuals, and male and female sex workers. These groups were divided into sub-groups of HIV positive and HIV negative participants. Twenty-five focus groups were held, with groups of 8-10 people in Lima/Callao and 4-5 people elsewhere. Forty-two in-depth interviews explored the information from the focus groups in greater depth. All activities used open-ended guides developed together with the collaborating organizations to discuss participants' perceptions regarding the risks of having sex, the sub-group with the highest risk, and the ways to diminish risks. Qualitative data were analyzed using the grounded theory approach to identify themes and categories, followed by coding. Participants also completed a survey about sexual practices, including perceived risks and use of prevention. Quantitative data were analyzed using STATA 9.0 to carry out bivariate analyses.

**Findings:** Qualitative results showed that all participants perceived two main risks when having sex: contract or become reinfected with HIV or contract another sexually transmitted infection. Members of all sub-groups affirmed that young people are at particularly high risk since they have multiple partners and do not use condoms, despite recognition that condom use and fidelity are two important means of protection. Quantitative results showed that despite perceiving group-level risks, almost one-third of participants perceived no or little personal risk to HIV. Results also demonstrated low condom use at last sex: 43% of participants reported use with female partners and 63% and 64% of participants reported use with male and transgendered partners, respectively.

**Contribution to Knowledge:** Youth participants certainly recognize the HIV-related risks of young people as a group. This recognition does not, however, translate into awareness of personal risk and use of preventive practices. Further research needs to explore how to make overall awareness translate into personal awareness and prevention.

# Let's Get Physical: Sexually Transmitted Infections and Body Mass Index among US Young Adults.

**Bitá Amani**

Public Affairs

Pervasive thin and muscular beauty standards persist within a societal context where maintaining and achieving a healthy lifestyle and diet has become increasingly difficult. Additionally, growing public health concern about dramatic increases in body mass index (BMI) may have impacted how individuals perceive and feel about their bodies. Possibly, sexually transmitted infection (STI) prevalence is influenced by this context. In order to investigate this possibility, this study examined the intersection of STIs and BMI. Data from the 1999-2004 National Health and Nutrition Examination Survey collected among 20-29 year old men and non-pregnant women was used to examine associations between STI, sexual behavior, and BMI. Associations between outcomes (STIs, number of partners, coitarche) and exposure (BMI) were examined using logistic regression. To assess associations within categories of BMI, piecewise-continuous linear models were employed. Analyses were stratified by gender. The study population was primarily non-Latino White with a mean age of 24.4 years. The prevalence of Herpes Simplex Virus-2 (HSV-2) was higher among women (19%) compared to men (8%). Prevalence of HSV-2 among underweight men was higher than normal weight men (OR=3.59, 95% CI 1.02-12.6) in adjusted analyses controlling for race/ethnicity, age, income, and lifetime partners. Among women, no associations between non-viral STIs and BMI were seen, but HSV-2 varied within categories of BMI. Specifically, among obese women, the odds of testing positive for HSV-2 increased as BMI increased. In terms of sexual behavior, women who were obese had greater odds of reporting <16 years age at first sex compared to women with normal weight. Also, obese women were 3.5 times (95% CI 1.7-7.1) more likely to report no sexual partners in the last year compared to normal weight women after adjustment for race/ethnicity, age, income, and marital status. Underweight men were more likely to report 0 partners in the last year compared to normal weight men (OR=3.3, 95% CI 1.1-10.2) after adjustment for the same potential confounders. Obese women had a greater odds of reporting >5 partners in the last year compared to normal weight women (OR=2.7, 95% CI 1.2-6.4). This analysis reveals possible disparities in STI prevalence and reported sexual behavior by BMI among men and non-pregnant women. Given these findings, further research into how STI and obesity are related is recommended. Additionally, targeted STI interventions aimed at addressing discourse on body size may be appropriate.

# Differences in Sexual Position Between Men Who Have Sex with Men (MSM) and Men Who Have Sex with Men and Women (MSMW)

Ryan Murphy

Epidemiology

**Rational:** To describe differences in sexual positioning between men who have sex with men (MSM) and men who have sex with men and women (MSMW) to determine its influence on STI and HIV transmission in Los Angeles. To identify if strategic positioning is occurring and the characteristics of those likely to practice it.

**Methods:** 690 males who participated in one of two waves of data collection (2005-2006; 2006-2007) at the Los Angeles site for NIDA's Sexual Acquisition and Transmission of HIV Cooperative Agreement Program (SATH-CAP) completed Audio Computer Assisted Self interviews, oral HIV rapid testing with confirmatory blood test by Western Blot and provided urine specimens for detection of powder cocaine, crack cocaine, methamphetamine and heroin use with the past 3 days. Recruitment was conducted using respondent driven sampling with each participant recruiting 0-6 drug using or sexual partners. MSM and MSMW were defined by gender of reported sex partners within the past 6 months. Sexual position was examined with up to 3 partners reported on and defined as oral-only, insertive, receptive and versatile anal (both insertive and receptive). Inclusion criteria for this analysis were MSM and MSMW who reported sexual positioning behaviors with a least one male partner in the past 6 months. Chi-square tests were used to test independence between these groups with regard to demographic characteristics, substance use, sexual behaviors and HIV status. Generalized linear random intercepts models were fit to predict sexual positioning.

**Results:** Participants were mostly middle aged (mean 41.8 years), minority, and lower income, with 83% earning \$1,000 or less in the previous month. About half the sample had been homeless at some point in their lives and 61% had been to jail or prison. Drug use was common in the sample and differed between MSM and MSMW. 11% of MSM and 3% of MSMW tested positive for methamphetamine and 13% of MSM and 24% of MSMW tested positive for cocaine. Overall HIV prevalence was 63% among MSM and 15% among MSMW. There were strong differences in sexual positions reported by MSM and MSMW. More MSMW reported oral-only sex with their male partners (43%) than MSM (18%). Among those reporting anal intercourse, more MSMW were insertive (66%) than MSM (38%) and fewer receptive (15%) than MSM (31%). MSMW (20%) were also less likely to be versatile than MSM (31%). In multivariate models, being HIV+ or reporting a partner who was Black was associated with lower odds of having unprotected insertive anal intercourse (UIAI), while those with an HIV+ partner had a higher odds of UIAI. MSMW (OR 0.39, 95%CI: 0.16-0.99) had a lower odds of unprotected receptive anal intercourse (URAI) compared to MSM and being HIV+ was associated with a higher odds of URAI.

**Conclusions:** MSM and MSMW differ in demographics, drug use practices and preferred sexual positions with male partners. More MSMW engage in oral-only sex and fewer practice URAI than MSM, reducing the risk of HIV acquisition. Participant and partner HIV status are strongly associated with sexual position. Positions with a higher risk of transmitting HIV are practiced less by participants who are HIV+ and positions with a higher risk of acquiring HIV are practiced less with partners who are HIV+, suggesting that both MSM and MSMW use strategic positioning to reduce the likelihood of transmitting or acquiring HIV.

# The Relative Role of Perceptions of Partner Risk in Unprotected Sex

**Allison Ober**

Social Welfare

**Background:** The effect of risk perceptions on condom use is well documented, but risk perception models often do not include perceptions of partner risk factors or important contextual variables which may affect inferences about risk perception.

**Methods:** Participants are women from three U.S. cities ( $n=1,267$ ) who reported on sexual episodes with recent sexual partners. We fit multilevel random effects logistic regression models to account for multiple episodes per subject.

**Results:** In univariate models predicting unprotected vaginal sex, women who perceived partners to be HIV positive (OR 0.44, 95% CI 0.21 - 0.94); bisexual (OR 0.51, 95% CI 0.37 - 0.65); or to have concurrent partners (0.55, 95% CI 0.43 - 0.71) were less likely to report unprotected sex. However, in a multivariate model that adjusted for contextual variables, partner risk perceptions did not decrease the odds of unprotected vaginal sex. Factors that did increase the odds of unprotected sex in the multivariate vaginal sex model included alcohol use during sex (AOR 1.64, 95% CI 1.10 - 2.46) and sex with a main partner (AOR 5.68, 95% CI 4.14 - 7.80). In univariate models predicting unprotected anal sex, women who perceived partners were bisexual or drug injectors were more likely to report unprotected anal sex. In a multivariate model for unprotected anal sex, perceived bisexuality was associated with increased odds of unprotected sex for women who reported using heroin in the past 30 days. Other factors associated with increased odds of unprotected anal sex included the woman's positive HIV status (AOR 5.15, 95% CI 1.44 - 18.32), sex with a main partner (AOR 1.96, 95% CI 1.16 - 3.31), alcohol use (AOR 1.95, 95% CI 1.06 - 3.58), and methamphetamine use (AOR 5.16, 95% CI 1.41 - 18.89).

**Conclusions:** When contextual variables are taken into account, the effect of partner risk perceptions on unprotected vaginal sex is not significant. Unprotected anal sex is associated with multiple risk factors, including the perception of partner bisexuality for women who use heroin. Findings have important implications for HIV prevention intervention with low-income, urban women of multiple races/ethnicities.

# Feasibility of voluntary testing and counseling for the partners of pregnant woman in prenatal care

**Nava Yeganeh**

Pediatric Infectious Diseases

HIV is a devastating preventable disease in pediatrics, resulting in increased morbidity and mortality in both infected and non-infected children worldwide. If infected, HIV positive children confront dire health outcomes with a 60% mortality rate in untreated children prior to their second birthdays. Even when not infected, some 15.2 million children around the world have lost one or both parents to AIDS, resulting in significant physical and emotional hardships, and making them vulnerable to abuse, exploitation and neglect. Given the lack of an effective vaccine and the complexity of life long treatment, prevention of maternal to child transmission of HIV continues to be a crucial public health measure in the area of child's health globally.

In Brazil, despite noteworthy achievements by the Brazilian National HIV/AIDS Program, HIV rates continue to rise in vulnerable sub-pockets of the population, including pregnant women. Previous studies from our group have shown that the prevalence of HIV in Porto Alegre, Brazil among women receiving prenatal care rose from 0.5% in 1997 to 3.5% in 2007. Furthermore, research done at this site and other sites show that women who become infected with HIV during pregnancy are more likely to transmit the infection to their infants than women who were infected prior to pregnancy. Thus, prenatal testing of the woman alone is not sufficient to circumvent risk of HIV transmission to infants.

Given this unfortunate pattern, we hypothesize that implementation of partner testing for HIV-1 in prenatal care is a feasible and viable measure to decrease the number of children affected by HIV both by decreasing maternal to child transmission and by improving the health of both parents. Specifically, we aim to (1) Evaluate the feasibility of preventing pediatric HIV infection by implementing partner HIV-1 VCT in prenatal clinics in Porto Alegre, Brazil. 2) Measure HIV-1 prevalence rates in partners of pregnant women receiving pre-natal care in Porto Alegre in order to increase antiretroviral uptake in this population thus decreasing sexual transmission. 3) Obtain reported sexually transmitted disease (STD) prevalence rates causing pediatric disease (such as syphilis) among study participants through administration of a structured survey. We aim to enroll a 1000 pregnant women over the age of 18 receiving prenatal care at Conceição Hospital, who are in a relationship > 3 months, as well as their partners. Measured outcomes include: (1) Proportion of pregnant women who know their partner's HIV serostatus at baseline and following intervention (2) Proportion of partners who accept HIV testing at baseline and at the end of the study. (3) HIV-1 prevalence among partners of participating women (4) Rates of congenitally acquired diseases such as syphilis among participants. (5) Proportion of HIV infected infants born to participants.

The strategy of testing male partners of women in prenatal care offers additional opportunities for reducing the risk of pediatric HIV infection in an area with high HIV prevalence, and can be extended to other congenitally acquired STD's, such as syphilis. Furthermore, the identification of HIV-infected parents with subsequent treatment with antiretroviral regimens can improve health outcomes for children directly by decreasing parental mortality, and even indirectly, as the initiation of anti-retroviral treatment of adult house members has been shown to result in increased school attendance. We believe that our study results will guide health policies in Brazil, where the Minister of Health has already shown interest, and in other countries with high HIV prevalence rates.

# Seeking Psychological Cover: The Relationship between HIV Testing, Health Services Usage, and Stigma

Sean Young

Medicine

We explore whether HIV stigma is associated with seeking to conceal testing interest. We examine 86,899 outpatient visits in a 1993-1997 national survey and compare HIV testing to 4 non-stigmatized tests: spirometry, allergy testing, mammography, and colonoscopy. We explore whether people testing for HIV, compared to people receiving control services, listed reasons for visit less related to the testing performed, listed their interest in testing more frequently as a non-primary reason for visit, and received more services unrelated to testing. A total of 48.7% of people testing for HIV listed a reason unrelated to testing as their primary reason for visit (spirometry: 8.9%; allergy testing: 29.3%), and 69.9% of people asking to test requested HIV testing as a secondary reason for visit (spirometry: 52%; allergy testing: 0%). People who tested for HIV received more services ( $M= 1.83$  additional services) than non-testers ( $M= 0.95$ ) on an index of 7 services. We did not find this association for spirometry, allergy testing, colonoscopy or mammography. We interpret these results to indicate that stigma may have behavioral correlates and that people may attempt to avoid HIV stigma by seeking a psychological cover for HIV testing. To our knowledge, this is the first study to attempt to use observational data on health service usage for assessing stigma and people's attempts to deal with HIV testing stigma.