

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

ROUTINE OFFER OF PROVIDER-INITIATED HIV TESTING AND COUNSELING FOR ALL TUBERCULOSIS PATIENTS IN INDIA: CHALLENGES AND RECOMMENDATION FOR NEW POLICY AND APPROACHES TO IMPLEMENTATION

**Authors and  
Affiliation:**

**Anupama Lochab**; Dr. Linda Rose Frank  
 Pennsylvania/Mid-Atlantic AIDS Education and Training Center, Department of IDM, GSPH

**Text:**

**Objectives:** To propose a policy recommendation to routinely offer HIV testing and counseling for all patients diagnosed with tuberculosis in India. India has a growing problem of HIV, TB and sexually transmitted infections. WHO/UNAIDS in 2006, had recommended implementation of provider initiated HIV testing and counseling in a phased manner in countries with generalized HIV epidemic and limited resources.

**Methods:** Published epidemiological studies on HIV and TB were reviewed for the incidence, prevalence and potential barriers to routine HIV testing and counseling. National AIDS Research Institute in India was consulted for latest HIV and TB surveillance reports. Studies presenting successes and failures of provider initiated HIV testing and counseling around the world were reviewed. WHO/UNAIDS 2006 guidelines were used to frame the proposed policy recommendations on routine HIV testing in tuberculosis patients in countries with limited resources.

**Results:** HIV/AIDS and tuberculosis are major public health challenges in India. The research data showed that there has been an increasing trend of HIV seropositivity among tuberculosis cases in the country. Tuberculosis is the most common opportunistic infection among those with HIV/AIDS in India. Studies from both resource poor and resource rich countries around the world, demonstrate success of routine HIV testing in health care facilities. There are also many challenges such as need of HIV and TB services integration, lack of HIV awareness and education, HIV stigmatization of TB services, need of more trained staff and funding, simultaneous expansion of antiretroviral treatment for HIV patients. Review of studies indicates that the important components of testing are 3C's confidentiality, consent and counseling.

**Conclusion:** The proposed policy to offer routine provider initiated HIV testing and counseling (PITC) for all TB patients, represent a major public health opportunity to mark the initiation of routine HIV testing and counseling in healthcare facilities in phased manner. This will also assist in appropriate utilization and acquisition of resources for HIV testing. Furthermore, it will inform new policy development and increase commitment to scale up the existing HIV prevention activities in the country.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

CANCER IN HIV POSITIVE INDIVIDUALS – Changing Trends and Public Health Implications-A Literature Review

**Authors and  
Affiliation:**

**Ashish Gupta**

Student- MPH (Community Health track), Department of Infectious Diseases and Microbiology, University of Pittsburgh, Graduate School of Public Health.

**Text:**

**Objective:** The objective of this review was to understand the changing trend of cancer in HIV and its public health implication.

**Methods:** A literature review of the studies on the changing trends of the cancer in HIV patients. These included studies on the cancer in the pre HAART and post HAART era. The review also includes studies on the incidence of non-AIDS malignancies like lung cancer, prostate cancer, colorectal cancer and breast cancer in HIV positive patients. The studies on cancer in elderly who are HIV positive were also a part of this review.

**Results:** The presence of malignancies in HIV positive individuals have been known since the beginning of the HIV epidemic. Kaposi's sarcoma and non-Hodgkin's lymphoma were the predominant malignancies in the pre HAART era. The trend has changed from higher incidence of AIDS defining malignancies to Non-AIDS defining malignancies over the years. These include a higher incidence of lung, prostate, colorectal and breast cancers. The analysis of various studies attributes this changing trend to the use of HAART in HIV positive individuals. Non AIDS related malignancies present clinically at a younger age, with a more aggressive behavior and at a more advanced stage in HIV positive individuals than in the general population. The more complex behavior of the two diseases as well as the lack of combined safety and pharmacokinetics of antiretrovirals and antineoplastics makes the task more difficult.

**Conclusion:** The public health implication of these co-morbidities needs to be evaluated as we have ever rising cases of both the diseases in the community. The expected global burden in terms of the disease mortality and the economic impact needs to be evaluated and preventive strategies need to be developed.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
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**September 17, 2008**

**Abstract  
Title:**

**DETECTION AND QUANTITATION OF HIV-1 IN THE FECES SAMPLES OF AIDS COHORT SOCIETY DONORS.**

**Authors and  
Affiliation:**

Ayan K. Chakrabarti, William G. Buchanan, Nathaniel J. Soltesz, Brian J. Golgan, Lori Caruso, Ming Ding, Phalguni Gupta, Charles R. Rinaldo and Yue Chen

**Text:**

**Department of Infectious Diseases and Microbiology, Graduate School of Public Health. University of Pittsburgh, PA, 15261**

**Background and Objectives:** HIV infects and depletes CD4+ T cells in Gut Associated Lymphoid Tissues (GALT) of GI tract at very early stages of infection. Furthermore, GALT are the major reservoirs of HIV-1 and may constantly shed virus and CD4 T cells into the intestinal lumen in the entire course of disease. We hypothesize that the dynamic change of HIV-1 and CD4 cell quantity in feces is linked to disease progression and can be used to predict the disease prognosis. Aims of this study are:

1. Establish the standard methods for quantitation of HIV-1 and CD4 RNA in feces.
2. Quantitation of HIV-1 and CD4 in RNA from feces samples of HIV infected subjects and its correlation with disease progression.

**Methods:** Feces were collected from consented normal and HIV-1 infected subjects from the Multicenter Cohort Study and stored in RNAlater solution at -80C until use. DNA and RNA were isolated from 200mg of the feces samples using nucleic acid isolation kits from Qiagen and Biomerieux respectively. Different concentration of HIV-1 positive 8E5 cells or HIV-1 positive serum was used to spike normal feces before isolation. PCR, RT PCR were performed using HIV-1, CD4 and beta-globin specific primers to quantitate viral and CD4 RNA contained in these feces samples.

**Results:**

From normal feces spiked with 8E5 cells, as low as 2.5 copies of HIV-1 DNA were detected per PCR reaction. From normal feces spiked with HIV-1 positive serum, 40 copies of HIV-1 RNA were detected per RT-PCR reaction. From normal feces samples, human beta-globin and CD4 RNA were also detected in 10mg feces.

From 10 frozen feces samples collected from HIV-1 positive patients in 2007, HIV-1 RNA was detected in feces in 2 out of the 6 patients with high viral load. Human beta-globin mRNA was detected in 25mg feces by RT-PCR.

**Discussion:** GALT is the largest lymphoid organ in the human body and main target for HIV-1 infection. Due to the anatomic location, little is known of immune function, cell infection and destruction of GALT during HIV-1 infection, which is vitally important for disease progression and therapeutic intervention. The HIV-1 RNA in feces may be cell-associated or free virus from infected GALT and human CD4 mRNA in feces could be the result of massive CD4 depletion in GALT. Thus it is highly possible that quantity of HIV-1 RNA and human CD4 mRNA in feces reflects HIV-1 associated pathogenesis in GALT and associated with disease progression.

**Conclusion:** Feces is a suitable specimen from which viral and human nucleic acids could be isolated and characterized

**Future direction:** Currently we are undergoing a cross sectional study using MACS donors from 4 different groups ; HIV negative, HIV+/HAART naïve, HIV+/Under HAART with undetectable viral load and HIV+/Under HAART with detectable viral load. The objective of this study is to perform a quantitative comparison between blood and feces with respect to viral load and CD4.

**CATEGORY OF EMPHASIS**

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Bioscience

Education/Prevention

**ABSTRACT FORM**  
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**September 17, 2008**

**Abstract  
Title:**

THE ROLE OF HUMAN GENE COPY NUMBER VARIATION IN LIPODYSTROPHY ASSOCIATED WITH HIGHLY  
ACTIVE ANTI-RETROVIRAL THERAPY

**Authors and  
Affiliation:**

**Bosko R, Martinson J and Kingsley L**  
 University of Pittsburgh Graduate School of Public Health, Department of Infectious Disease and Microbiology

**Text:**

Description of Problem: HIV infection for many is accompanied by alterations in lipid and glucose metabolism that can be further intensified by antiretroviral therapy in some individuals resulting in a condition defined as lipodystrophy. As not everyone on antiretroviral therapy exhibits such alterations, genetic factors likely play a role. More recently it has been observed that many genetic variations alter the amount of gene product produced, a quantitative effect, rather than changing the functionality of that product. Such mutations are not only seen as single nucleotide polymorphisms (SNPs) in transcriptional control regions but also as gene copy number variation (CNV). Because this variation can alter gene product levels, it is important to study the effect of CNV on genes related to lipid and glucose metabolism for a role in lipodystrophy.

Approach Taken: Copy number variation will be determined by multiplex amplifiable probe hybridization (MAPH) on a cross-sectional sampling of 2,534 individuals from the Multicenter AIDS Cohort Study (MACS) broken into cases with high LDL/low HDL and matched controls with low LDL/high HDL. Seventeen genes already shown to be associated with lipid metabolism will be initially studied. Those identified to have CNV will be analyzed for gene expression by quantitative real time reverse transcriptase PCR. To determine the effects of copy number on SNP genotyping, high frequency SNPs in high copy number regions will be selected using Hapmap. Denaturing High Performance Liquid Chromatography (DHPLC) of these SNPs will be used to identify CNV and Fluorescence Polarization (FP) will be performed to identify if the CNV observed by the DHPLC has an effect on standard SNP genotyping.

Data Analysis/Results: We have been able to successfully hybridize MAPH probes to membrane bound DNA, retain bound probes during washes and recover probes from the membrane. Currently, we are in the process of optimizing the post recovery probe amplification. Once CNV is determined for each gene, we will compare observations between the cases and controls. We have also been successful at generating FP data and upon optimization of the DHPLC; we will perform Cluster Analysis to identify the role of CNV in SNP genotyping.

Implications: By understanding the affect gene copy number plays in the human genome, we can identify targets of further research and new therapies along with risk factors for lipodystrophy in HIV+ patients. We can also determine what role genotyping risk factors will play when multiple copies of a gene can mask the risk associated with a certain genetic factor.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
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**September 17, 2008**

**Abstract  
Title:**

**AN INVESTIGATION INTO THE PATTERNS AND LEVELS OF EXPRESSION OF THE HOMEOSTATIC CHEMOKINE, CXCL13, DURING THE COURSE OF SIMMIAN IMMUNODEFICIENCY VIRUS INFECTION IN LYMPHOID TISSUES OF CYNOMOLGOUS MACAQUES.**

**Authors and  
Affiliation:**

**Carissa M. Flores, Fallert-Junekko, B., Cillo T., Qin S., Sui Y., and Reinhart, T.A., University of Pittsburgh, School of Public Health, Department of Infectious Disease and Microbiology**

**Text:**

Description of the problem: Homeostatic chemokines and their receptors direct trafficking of lymphocytes to the lymphoid organs. CXCL13 is a member of the homeostatic chemokine family and with its receptor, CXCR5, directs homing of dendritic cells, B and T cells to the germinal centers of those lymphoid tissues. The mechanisms by which CXCL13 and other homeostatic chemokines operate can be manipulated and exploited by pathogens such as the simian immunodeficiency virus (SIV) for their own benefit and survival. The exact processes these pathogens utilize to take advantage of the chemokine network remain uncertain and are an area of active research.

Methods: In this study, we sought to determine histologically the presence of CXCL13 mRNA and protein in mesenteric lymph node (MLN) and spleen tissues of uninfected, acutely SIV infected or AIDS-developing cynomolgous macaques (*Macaca fascicularis*). In addition, we investigated the potential genetic diversity in the *cxcl13* gene of cynomolgous, rhesus and pigtailed macaques that were also among the three disease states.

Results: Our results provide evidence that there are distinct and differing localization patterns of CXCL13 mRNA and protein in germinal centers of both lymph node and spleen tissues. These patterns are visually distinct between disease states, with AIDS tissues having an apparent mRNA pattern that differs from uninfected or acutely infected tissues. Single nucleotide polymorphisms were found in the *cxcl13* gene among all three species of macaques that resulted mostly synonymous changes in the amino acid sequence of CXCL13; however they were not numerous enough thus far to lead us to investigate their role in the pathogenesis of SIV.

Implications: The differences in the presence of CXCL13 mRNA and protein in AIDS tissues versus uninfected or acutely infected tissues have prompted more questions about whether this chemokine as well as other homeostatic chemokines and their receptors, may role in the pathogenesis of SIV. The next phase of this investigation will lead us to compare the patterns and levels of expression of all homeostatic chemokines in lymphoid tissues of cynomolgous macaques and determine their possible colocalization with lymphocytes or DCs, and SIV, potentially revealing changes to host processes during SIV infection.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
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**September 17, 2008**

**Abstract  
Title:**

USING MATURE DENDRITIC CELLS TO ENHANCE T-CELL RESPONSES AND INCREASE EPITOPE DETECTION IN HCV-INFECTED SUBJECTS.

**Authors and  
Affiliation:**

Catherine M Howe

**Text:**

**Description of the Problem:**

Hepatitis C virus (HCV)-specific T-cell responses are strong and multi-specific during the acute infection phase, and may play a significant role in viral clearance. However, these responses are not maintained in most patients who later develop a chronic infection, which is characterized *in vitro* by weak or undetectable HCV-specific T-cell responses. Dendritic cells (DCs) are potent antigen presenting cells (APCs) that prime and activate T-cells. We have previously shown that mature dendritic cells (mDCs) are able to boost T-cell responses in chronic human immunodeficiency virus (HIV) infection. Since HCV is similar to HIV in its variability and ability to set up a chronic infection, we propose it would respond likewise to the influence of DCs. We hypothesize therefore that mDCs from HCV-infected patients are able to enhance HCV-specific T-cell responses and increase the breadth of HCV epitopes detected compared to using immature DCs or peptide stimulation alone. Assessing the role of DCs in HCV-specific immune responses will benefit our understanding of HCV pathogenesis and provide insights into development of therapeutic DC vaccine strategies.

**Methods:**

We will determine the ability of mature and immature DCs to enhance T-cell response to 9-mer HCV-specific peptides using an ELISPOT assay measuring IFN- $\gamma$  production. We will then assess mDCs' capacity to increase sensitivity in epitope discovery studies using 18-mer overlapping peptides in an ELISPOT assay. We will finally conclude by characterizing the epitope-specific T-cell responses using ICS and CFSE proliferation assays.

**Results:**

Our preliminary data indicated that there are two contrasting patterns in DC function in HCV infection. In some HCV infected subjects mature DCs were able to enhance T-cell responses to HCV-specific peptides, while in other subjects mDCs were unable to boost T-cell responses, and were even less functional when compared to immature DCs (iDCs). The experiments will be repeated to confirm our results.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
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**September 17, 2008**

**Abstract  
Title:**

**Title: Development of a Murine System to study the modulation of the CXCR3-ligand interaction**

**Authors and  
Affiliation:**

**Authors: Charis Tjoeng, Shulin Qin, Kristi Gaus, Todd Reinhart**

**Text:**

**Text:**

The body's immune response to a microbial pathogen can play a major role in determining disease outcome. Chemokines and chemokine receptors modulate the immune response by recruiting specific immune cells to sites of inflammation. For example, one of the functions of chemokine receptor CXCR3 and its ligands is to regulate trafficking of activated T cells to sites of infection and inflammation. The ability to manipulate chemokine-chemokine receptor interactions may serve as a valuable tool when developing therapeutics to aid the host against certain pathogens. I aim to develop a model to study the modification of the murine CXCR3-ligand interaction by various agents. Specifically, I will test the hypothesis that extracellular loop peptide mimetics of CXCR3 will block CXCR3 ligand function. Currently I am cloning the murine CXCR3 receptor cDNA into murine L1.2 cells, and I plan to perform chemotaxis assays to determine how the interaction is affected by CXCR3 extracellular domain peptides. These findings will be compared to studies of chemokine responsiveness of primary mouse T cells. This work will facilitate the transition from in vitro to in vivo studies to better evaluate how chemokine-chemokine receptor interactions can be exploited for therapeutic use.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**GENETIC CHARACTERISTIC OF HIV-1 IN SEMEN AND  
BLOOD FROM INDIAN INFECTED SUBJECTS**

**Authors and  
Affiliation:**

**Shen, Chengli**<sup>1</sup> Ding, Ming<sup>1</sup> Craigo, Jodi K<sup>2</sup> Gupta, Phalguni<sup>1</sup>  
 1.Infectious disease and microbiology Graduate School of Public Health University  
 of Pittsburgh 15261 2.Microbiology & Molecular Genetics University of Pittsburgh  
 School of Medicine 15261

**Text:**

Human immunodeficiency virus type 1 (HIV-1) in the male genital tract contains virus formed locally in addition to virus imported from the circulation. Due to tissue-specific environment and immunological pressures, Virus produced in the male genital tract may be genetically distinct. This study is to determine the biologic properties of sub type C HIV-1 between semen and blood in subtype C-infected subjects in Indian.

Paired Seminal plasma, seminal cells, blood plasma, and blood cells from HIV-1 Indian-C positive subjects were processed for RNA isolation. 15 to 20 nucleic acid sequences of the C2-V5 were obtained from each compartment of each subject. Shannon entropy analysis showed that C2, C3, V4, V5 are more variable and V3 region is more conservative, but the flanks of V3 region, C2 and C3 regions are more variable than V3 region. The Slatkin and Maddison and Gene Flow test confirmed that four of the five patients have distinct compartmentalization. In addition, immune selection analysis showed that in blood part, V3 region is under positive selection and in semen part, all of C2, V3, C3 regions are under positive selection. Interestingly, all the viral population were exhibited CCR5 coreceptor usage, and non- syncial inducing and no special pattern of N-linked glycosylation among the four compartments were found. This study is very important for understanding the long-term response to antiretroviral therapy, the design of vaccines, and the sexual transmission of HIV.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**DEVELOPMENT OF A ROBUST SYSTEM OF STUDY OF CHEMOKINE CCL20 FUNCTION**

**Authors and  
Affiliation:**

**Cynthia R. Klamar**<sup>1</sup>, Shulin Qin<sup>1</sup> and Todd A. Reinhart<sup>1</sup>

<sup>1</sup>Department of Infectious Disease and Microbiology, University of Pittsburgh, PA 15261

**Text:**

Chemokines are chemoattractant proteins that play multiple roles in the immune system, including modifying the host response to infection. One particular chemokine, CCL20 and its receptor CCR6, is thought to be an integral part of the communication between the innate and adaptive arms of the immune system. Expression of the receptor on immature dendritic cells has provided impetus for study of the response to this chemokine in vaccination. During some viral (SIV, HIV, and HTLV), bacterial (*Salmonella*, and *Helicobacter*) infections, as well as during an immunological response to some cancers, CCL20, including its receptor CCR6, have been shown to be upregulated in expression. More recently, the highly studied Th17 subset of T cells have been shown to also express the receptor for CCL20. Therefore, CCL20's role during infection and homeostasis of the immune system should be elucidated. Obtaining chemokines for study in vaccination and other immunological experiments can be costly. Additionally there are few, if any, commercially available non-human primate chemokines for use in immunological study models with close relation to the human. Purification of a recombinant, potentially bioactive non-human primate chemokine of interest from a bacterial expression system could be a flexible and cost effective way to obtain chemokines for vaccination and immunological studies. Additionally, peptide synthesis can provide large quantities of pure, correctly refolded protein for experimental and therapeutic use by way of regioselectively cyclized peptide synthesis. The objective of these studies has been to develop a system for studying the CCL20: CCR6 (chemokine:chemokine receptor) interaction. Additionally, it has been to demonstrate that rhesus macaque chemokine CCL20 can be purified following expression from an *E.coli* plasmid vector in abundance and to a high degree of purity. Data will be presented on the expression system and its use to date in obtaining purified, recombinant CCL20. Additionally, data will be presented characterizing the bioactivity of regioselectively refolded rhesus CCL20 in comparison to the commercially available human chemokine through transiently and stably transfected cell lines. Comparative analysis of the amino acid sequences of the chemokines will be presented to potentially explain the differences of bioactivity. Further study will be done to optimize the analysis of the developed system for studying non-human primate chemokine: chemokine receptor interactions, including flow cytometry and possibly chemotactic inhibition studies. Additionally, optimization and further study of the *E.coli* expression system for obtaining recombinant chemokines will be performed.

**CATEGORY OF EMPHASIS**

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Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**CYTOKINE INDUCTION BY HHV-8 IN MYELOID DENDRITIC CELLS AND B CELLS**

**Authors and  
Affiliation:**

**Emilee Knowlton**, Giovanna Rappocciolo, Paolo Piazza, Heather Hensler, Frank J. Jenkins, Mariel Jais, Charles R. Rinaldo  
Dept of Infectious Diseases and Microbiology, Graduate School of Public Health,  
University of Pittsburgh, PA

**Text:**

**Objectives:** Myeloid dendritic cells (DC) and activated B lymphocytes are professional antigen presenting cells susceptible to HHV-8 infection following DC-SIGN receptor binding. However, the virus undergoes productive infection in B cells but not in DC. We examined whether these different pathways taken by the virus were related to differential cytokine production in B cells and DC.

**Methods:** Monocyte derived DC and activated B cells were infected with purified HHV-8 and supernatants were examined for cytokines at 0, 6, 12, 18, 24, and 48 hours post-infection by the Cytometric Bead Array (BD).

**Results:** Peak levels of RANTES, TNF- $\alpha$ , IL-6, IL-8, IL-10, MIP-1 $\alpha$  and MIP-1 $\beta$  of 52 (IL-10) to  $1.6 \times 10^4$  (MIP-1 $\beta$ ) pg/ml were detected in B cell cultures by 24-48h of infection, which were 4 to 32 fold higher than in uninfected B cell cultures. In contrast, DC produced peak amounts of MCP-1, MIP-1 $\alpha$  and MIP-1 $\beta$  of 234 (MCP-1) to  $1.0 \times 10^4$  (MIP-1 $\beta$ ) pg/ml by 24-48h, which were 2.4 to 20 fold higher than in uninfected DC, and did not produce higher levels of IL-8, IL-12p70 or TNF compared to uninfected DC.

**Conclusions:** Elevated levels of several inflammatory cytokines and MIP chemokines were detected in HHV-8 infected B cells. In contrast, HHV-8 infected DC produced chemokines, but not IL-12 p70. Distinct cytokine production by HHV-8 infected B cells and DC could play a role in viral replication and functional abilities in these antigen presenting cells.

**CATEGORY OF EMPHASIS**

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Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract Title:**

**THE ROLE OF MIR-17-92 MICRORNAS IN TYPE-1 T-CELL RESPONSE TO CANCER**

**Authors and Affiliation:**

*Gary Kohanbash , Kotaro Sasaki, Ryo Ueda, Mitsugu Fujita, Xinmei Zhu, Edward Kastenhuber, Heather Mcdonald, Walter Storkus, Hideho Okada.*

**Text:**

**Description of the problem.** The balance between health and disease remains in constant limbo with disease progression representing immune failure. This immune failure can occur either indirectly where the agent of disease shields itself from immune surveillance or directly, where the host immune system is manipulated to work in a way promoting the disease. It is well known that tumor derived factors directly skew T-cell differentiation from an effective tumor fighting Th1 state to an ineffective Th2 state, allowing for tumor growth. Therefore, we examined differences between these two states in order to develop a method for reverting the balance favoring the immune system, restoring health.

**Approach taken.** MicroRNAs (miRNAs) are a novel class of small non coding RNAs that have the capacity to control production of proteins by binding to mRNAs and inhibiting translation. Recently, the importance of miRNAs has been implicated in many diseases such as cancer, autoimmunity, and viral infections. We thereby sought to examine the differential expression of miRNAs in Th1 versus Th2 cells and the effect of these miRNAs on the specific phenotypes.

**Results.** MicroRNA microarray analyses revealed that miR-17-92, a miRNA cluster, was preferentially expressed in in-vitro induced murine Th1 cells versus Th2 cells. Quantitative RT-PCR further confirmed that miR-17-92 expression was consistently higher in Th1 cells than Th2 cells. Addition of neutralizing anti-IL-4 mAb during the culture period prevented the loss of miR-17-92 expression, indicating that IL-4 played a major role in suppression of miR-17-92 expression. Consistently, we observed that CD4<sup>+</sup> T cells deficient in Stat6, a critical component of the IL-4R signaling cascade, retained high levels of miR-17-92 expression, regardless of culture conditions used to polarize Th cells. MiR-17-92 expression correlated with differential proliferation capacity as Th1 cells proliferated at higher levels than Th2 cells and expressed lower levels of anti-proliferative(in T-cell) transcription factors E2F1 and E2F2, which are the known targets of miR-17-92. In-vivo experiments demonstrated that splenic CD4<sup>+</sup> and CD8<sup>+</sup> T cells obtained from B16 tumor-bearing mice showed the down-regulation of miR-17-92 expression and a Th2 phenotype including a decrease in both proliferation and IFN- $\gamma$  production, as compared with control mice. Finally, we demonstrated that CD4<sup>+</sup> and CD8<sup>+</sup> T cells in glioma patient showed a significant decrease in miR-17-92 cluster than those in healthy donors, which is consistent with the results described above.

**Conclusions.** Collectively, our data suggests that the type-2 skewing tumor microenvironment induces the down-regulation of miR-17-92 expression in T cells, thereby diminishing the effective proliferation of tumor-specific T cells and tumor destruction. We propose that viral therapy targeting this cluster may provide enhanced T-cell function in preventing tumor growth and in ameliorating many other diseases such as autoimmunity and viral infection through boosting the proper immune response.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

## ABSTRACT FORM

### Annual IDM Research Day

### September 17, 2008

**Abstract  
Title:**

**HEMATOLOGICAL AND INFLAMMATORY MEDIATOR ANALYSES IN KENYAN CHILDREN WITH *PLASMODIUM FALCIPARUM* AND BACTEREMIA CO-INFECTION FROM A HOLOENDEMIC MALARIA REGION**

**Authors  
and  
Affiliation:**

**Gregory C. Davenport<sup>1</sup>, Tom Were<sup>2</sup>, Collins Ouma<sup>2</sup>, James B. Hittner<sup>3</sup>, Yamo Ouma<sup>2</sup>, John M. Ong'echa<sup>2</sup>, and Douglas J. Perkins<sup>1,2</sup>**

<sup>1</sup> Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

<sup>2</sup> University of New Mexico/KEMRI Laboratories of Parasitic and Viral Diseases, Centre for Vector Biology and Control Research, Kenya Medical Research Institute, Kisumu, Kenya

<sup>3</sup> Department of Psychology, College of Charleston, Charleston, SC, USA

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<sup>5</sup> Department of Internal Medicine, Division of Infectious Disease, University of New Mexico, Albuquerque, NM, USA

**Text:**

**Objective:** Although blood-borne bacterial infections are suspected to enhance malarial disease severity, the role of inflammatory mediators in conditioning disease outcomes has not been reported. Children enrolled in our Severe Malarial Anemia study from a holoendemic region of western Kenya showed no exacerbation of anemia when malaria was complicated with concurrent bacteremia. However, a significant decrease in parasitemia was noted in children co-infected with either Gram (G)[+] or [-] bacteremia. Our objective was to determine the inflammatory mediator profile milieu associated with the hematological and parasitemia differences found in children co-infected with malaria and bacteremia.

**Methods:** HIV-1 negative children (aged <3yrs) infected with *P. falciparum* malaria were divided into parasitemic (n=156), G[-] bacteremia co-infected (n=22), and G[+] bacteremia co-infected (n=14) groups. In addition, a cohort of afebrile healthy children without parasitemia and hemoglobin (Hb) >11g/dL were included as controls (n=14) to detect depressed cytokine levels associated with infection. Since a pro-inflammatory milieu is known to control parasitemia, but adversely affect erythropoiesis, we performed a bead-based Multiplex assay on the plasma of these groups to determine the relative levels of 16 soluble mediators.

**Results:** While the median parasitemia for the G[+] and G[-] co-infected groups were nearly identical, only the G[-] group had a statistically significant decrease ( $P<0.001$ ) compared to the parasitemic group. Hematologically, both bacteremia co-infected groups had lower MCHC and increased granulocytes relative to the malaria-only group. As expected, general immune activation was noted in both co-infected groups. Compared to the parasitemic group, the G[-] co-infected group had elevated levels in IL-1 $\beta$  ( $P=0.009$ ), IL-1Ra ( $P=0.001$ ), IL-4 ( $P<0.001$ ), IL-5 ( $P<0.001$ ), IL-7 ( $P<0.001$ ), IL-12 ( $P<0.001$ ), IL-15 ( $P<0.001$ ), IL-17 ( $P=0.016$ ), IFN- $\alpha$  ( $P<0.001$ ), and IFN- $\gamma$  ( $P<0.001$ ), while lower levels were noted in IL-10 ( $P=0.016$ ) and TNF- $\alpha$  ( $P=0.030$ ). The G[+] co-infected group followed a similar pattern to the G[-] co-infected groups, with increases relative to the malaria-only group seen in IL-4 ( $P<0.001$ ), IL-5 ( $P<0.001$ ), IL-7 ( $P<0.001$ ), IL-12 ( $P=0.034$ ), IL-15 ( $P<0.001$ ), IL-17 ( $P=0.003$ ), IFN- $\alpha$  ( $P<0.001$ ), and IFN- $\gamma$  ( $P=0.008$ ), in addition to a decrease in TNF- $\alpha$  levels ( $P=0.004$ ). Only four differences were found between the G[+] and G[-] co-infected groups, with the G[-] co-infected children having the greatest concentrations in WBC count ( $P=0.026$ ), Granulocytes ( $P=0.007$ ), IFN- $\gamma$  ( $P=0.006$ ), and GM-CSF ( $P=0.025$ ).

**Discussion:** As expected, the co-infected groups showed increased levels in nearly all statistically significant analytes when compared to the malaria-only group. However, two key molecules, TNF- $\alpha$  and IL-10, known to contribute to SMA, were lower in these groups, and may explain how these groups have lower parasitemia while anemia is not worsened. In addition, the IFN- $\gamma$  levels of the G[-] co-infected group were greater than the malaria-only and G[+] co-infected group, which may also be driving the type 1 immune response resulting in lower parasitemia. By examining the inflammatory response pattern in malaria mono- and co-infection, and associating them with anemia and parasitemia, we can begin to elucidate the complex inflammatory mediator profile responsible for control of infection while preserving hemoglobin levels.

#### CATEGORY OF EMPHASIS

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**Functional consequences of altered oligomerization of HIV-1 Vpr alleles derived from Long Term Non-Progresser HIV-1 patients: Implications for designing new therapeutics targeting HIV viral proteins.**

**Authors and  
Affiliation:**

**Narasimhan J Venkatachari<sup>1</sup>, Danielle McKeithen<sup>1</sup>, Timothy M Dempsey<sup>1</sup>, Krisztina Baglyas<sup>1</sup>, Murali Ramachandran<sup>2</sup>, Alagarsamy Srinivasan<sup>3</sup> and Velpandi Ayyavoo<sup>1</sup>**

<sup>1</sup> Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261; <sup>2</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 <sup>3</sup>Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107.

**Text:**

**HIV-1 Vpr, a non-structural protein, is unique for being incorporated in the virus particle and possesses several characteristic features that are known to play important roles in HIV-1 replication and disease progression. Vpr has a positive role in efficient transport of PIC into the nucleus of non-dividing cells and enhance virus replication in primary T cells. To gain a better understanding of Vpr's role in pathogenesis *in vivo*, we used well-defined HIV-1 positive long-term nonprogressors (LTNP) from MACS cohort to identify Vpr alleles that might provide information on Vpr-mediated HIV-1 pathogenesis. Results from these studies indicate that Vpr alleles present in these patients have distinct mutations in Helix II and the C-terminal region, that are known to regulate Vpr oligomerization, virion incorporation and Vpr-mediated cellular events. Preliminary results indicate that transcomplementation of HIV-1ΔVpr virus with LTNP-Vpr allele significantly reduce the amount of virus produced as evaluated by single round infection assay and estimation of p24 released in the supernatant by ELISA. The reduction in the viral titer can be attributed to decreased LTR activity and sequestration of HIV-1 Gag in the infected cells in presence of hyperoligomeric Vpr alleles. Evaluation of Vpr oligomerization in live cells by Bimolecular Fluorescence complementation (BiFC) assay suggests abnormal oligomerization function for Vpr alleles present in LTNP. Additional work is in progress to simulate similar phenomenon with wild type Vpr protein during HIV infection, this will help us to gain information for future drug discovery targeting other viral proteins.**

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**Q509L in HIV-1 RT INCREASES AZT RESISTANCE BY PROMOTING POLYMERASE-COMPETENT VS. RNASE H-COMPETENT BINDING ON RNA/DNA T/P WITH SHORT DUPLEX LENGTHS**

**Authors and  
Affiliation:**

**J Brehm**, J Mellors, N Sluis-Cremer  
 University of Pittsburgh, Pittsburgh, PA

**Text:**

**OBJECTIVES:** A371V and Q509L were selected by AZT in combination with TAMs (D67N/K70R/T215F) and increase AZT resistance 50-fold compared to TAMs alone. Initial biochemical studies show that TAMs/Q509L and TAMs/A371V/Q509L increase AZT-monophosphate (AZT-MP) excision from RNA/DNA template/primers (T/P) by decreasing secondary RNase H cleavage events that reduce the RNA/DNA T/P duplex length to less than 12 nucleotides (nt). However, the precise mechanisms responsible for the decreased RNA cleavage and increased AZT-MP excision have not been defined.

**METHODS:** RT containing D67N, K70R, T215F, A371V and/or Q509L was over-expressed and purified. The rates for RNase H cleavage of AZT-MP terminated RNA/DNA T/P were determined using transient or steady-state kinetic approaches, both in the absence and presence of a nucleic acid trap. The ability of the wild-type or mutant RTs to bind RNA/DNA T/P with duplex lengths less than 18nt in a DNA polymerase- or RNase H-competent mode was assessed by measuring DNA polymerization or RNase H cleavage at defined times after the addition of trap to a pre-formed RT-T/P complex.

**RESULTS:** Initial RNase H cleavages for all enzymes were similar, suggesting that A371V and Q509L do not directly affect the catalytic activity of the RNase H active site. However, the rates for the subsequent RNase H cleavages, which occur after T/P dissociation and rebinding, were reduced 2.2- and 2.3-fold for the TAMs/Q509L and TAMs/A371V/Q509L RTs, respectively. RT-T/P binding assays showed that the Q509L mutation promoted RT binding to short T/P duplexes in a polymerase-competent mode favoring AZT-MP excision, rather than an RNase H-competent mode allowing additional cleavages and dissociation of the T/P complex.

**CONCLUSIONS:** The Q509L mutation does not have a direct effect on RT RNase H catalytic activity, but increases AZT resistance by promoting RT binding to RNA/DNA T/P duplexes <18 nucleotides in a polymerase-competent mode that favors excision rather than an RNase H-competent mode that favors further cleavage and T/P dissociation. These findings provide new insights into the mechanism by which mutations in the C-terminal domain of RT confer NRTI resistance.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

ROLE OF THE N348I MUTATION IN HIV-1 REVERSE TRANSCRIPTASE IN ZIDOVUDINE/NNRTI DUAL RESISTANCE

**Authors and  
Affiliation:**

**Jessica Radzio**<sup>1,2</sup> and Nicolas Sluis-Cremer<sup>2</sup>

<sup>1</sup>*Department of Infectious Diseases and Microbiology, University of Pittsburgh Graduate School of Public Health and*

<sup>2</sup>*Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA*

**Text:**

The efficacy of combination antiretroviral therapies for HIV-1 infection that include zidovudine (AZT) and nonnucleoside reverse transcriptase (RT) inhibitors (NNRTIs) can be explained by the observed synergistic interactions between these two classes of drugs as well as the antagonism between their respective resistance mutations (e.g. K70R for AZT; Y181C for nevirapine or etravirine). Recently, a novel mutation in the connection domain of HIV-1 RT, N348I, was found to co-associate with K70R and Y181C and to confer low-level resistance to both classes of drugs. In this regard, we hypothesized that the selection of N348I in HIV-1 RT may provide an alternate pathway to AZT/NNRTI resistance or, alternatively, could counteract the antagonistic interactions between K70R and Y181C. To address this, we investigated the ability of recombinant purified wild-type (WT), K70R, Y181C, N348I, K70R/Y181C, K70R/N348I and Y181C/N348I HIV-1 RT to carry-out DNA synthesis in the presence of AZT, NNRTI (nevirapine, efavirenz and etravirine) or combinations of both classes of inhibitors. The results demonstrate that K70R RT was the most efficient enzyme in excising AZT-monophosphate from DNA/DNA template/primers (T/Ps) whereas K70R/N348I RT was the most efficient on RNA/DNA T/Ps. By comparison, the Y181C and K70R/Y181C enzymes were less efficient than the WT enzyme on both T/P substrates. Surprisingly, Y181C/N348I RT could excise AZT-MP more efficiently than WT RT on RNA/DNA T/P, suggesting that N348I partially compensated for the poor excision activity of enzymes containing the Y181C mutation. Additional mechanistic studies suggest that this may be due, in part, to changes in the enzyme's RNase H activity. Furthermore, Y181C/N348I RT conferred the highest level of resistance to nevirapine, efavirenz and etravirine and carried-out efficient DNA synthesis when both AZT and NNRTIs were included in the assay. By contrast, enzymes that contained the K70R mutation were hypersusceptible to NNRTI-inhibition. Taken together, these results suggest an important role for N348I in conferring AZT/NNRTI dual resistance.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

DEVELOPING AN HIV/AIDS EDUCATION AND PREVENTION PROGRAM IN A LOCAL AFRICAN AMERICAN CHURCH

**Authors and  
Affiliation:**

**Kristina R. Williams**, MPH Program, Department of Infectious Diseases and Microbiology,  
University of Pittsburgh, Graduate School of Public Health.

**Text:**

The African American Community has been affected by Human Immunodeficiency Virus (HIV) and AIDS (Acquired Immunodeficiency Syndrome) for decades. Currently, Black Americans make up about 13% of the population, but account for nearly half of new HIV cases. Current prevention and intervention strategies targeting this population have had minimal results, as the rate of HIV infection in black men and women is approximately eight times greater than that of their white counterparts. Traditionally, the Black Church has served as the source of information and services, concerning religious and spiritual needs as well as health, financial, and social needs. The high incidence and prevalence of HIV/AIDS and the dependence upon the church creates a unique opportunity in which information concerning HIV/AIDS epidemiology, prevention strategies, and available services can be delivered to the targeted population. To address the HIV epidemic in the African American community, Wesley Center AME Zion Church developed an educational HIV Ministry. Members of the congregation were provided with HIV statistics and basic HIV/AIDS epidemiology, relevant to Black Americans, through bulletin inserts and posters. Additionally, the HIV ministry partnered with the Allegheny County Health Department to provide free HIV testing during the church's Annual Community Day, thereby making the community aware of the seriousness of the disease. In the first year, 43 people from the Hill District community were tested for HIV; 27 were tested the following year. HIV awareness and education will be continued and expanded to include seminars and workshops, collaborations with local churches and organizations, and prayer for those infected and affected by HIV/AIDS.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

Polymorphisms in the IL-12 gene in a Kenyan Pediatric Population may alter Plasmodium  
Falciparum Disease Outcomes

**Authors and  
Affiliation:**

**Strong L, Martinson J**

**Text:**

**Description of Problem:** *Plasmodium falciparum* malaria is a major disease in sub-Saharan Africa yielding a high mortality rate in children. The disease outcome varies between individuals and research has shown that this may be due to the particular genetics of the host. In the case of malaria, IL-12 has been identified as an important gene that may alter the disease outcome.

**Methods:** A case-control study will be used to investigate 4 Single Nucleotide Polymorphisms (SNPs) of the IL-12 gene and the IL-12 receptor gene. We will be using Fluorescent Polarization and the TaqMan assay to identify the SNPs in genes of approximately 800 DNA samples from malaria infected pediatric patients. These SNPs have been linked to either a protection for or a higher susceptibility to either high density parasitemia or severe malaria anemia.

**Data Analysis/Results:** T-tests will be used to compare allele frequencies of the SNPs. Conditional logistic regression will be used to analyze the different variables (high density parasitemia and severe malaria anemia). When all the factors are analyzed together we can come up with parameters that will predict disease outcome.

**Implications:** By investigating the influence that these alleles and factors have on patients with malaria, we could develop parameters that will predict the disease outcome of pediatric patients with malaria.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

CYTOKINE RESPONSES BY CD8 T LYMPHOCYTES TO HHV-8/KSHV LYTIC AND LATENCY PROTEINS

**Authors and  
Affiliation:**

**Lauren Lepone**, Giovanna Rappocciolo, Emilee Knowlton, Paolo Piazza, Mariel Jais, Frank J. Jenkins and Charles R. Rinaldo  
 Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh PA

**Text:**

**Objectives:** CD8 T cells producing more than one cytokine or cytotoxicity factor, i.e. polyfunctional, have been shown to relate to control of HIV-1 infection. Based on our previous findings that dendritic cells (DC) are optimally required as antigen presenting cells to reveal MHC class I epitopes of HHV-8/KSHV glycoprotein B (gB) (Blood 99:3360, 2002), we determined CD8 T cell cytokine responses to multiple lytic and latency viral proteins.

**Methods:** T cells were stimulated with DC from HHV-8 seropositive, HLA A\*0201 donors that had been loaded with overlapping peptides derived from gB and K8.1 lytic proteins, and K12 and latency associated nuclear antigen-1 (LANA-1) latency proteins, and screened for IFN $\gamma$  production by ELISPOT. Positive peptides were assessed for IFN $\gamma$ , IL2, TNF $\alpha$ , MIP1 $\beta$ , and CD107a production by flow cytometry to investigate the role of polyfunctional responses.

**Results:** ELISPOT and flow cytometry revealed new, potential HLA A\*0201 9mer epitopes for gB (5), K8.1 (4), K12 (2) and LANA-1 (5). In HHV-8 seropositive healthy donors controlling infection, the predominant CD8 T cell response to protein epitopes was monofunctional, with a portion of CD8 T cells being polyfunctional.

**Conclusions:** This demonstrates that HHV-8 proteins predominantly induce single cytokine or cytotoxicity factors in CD8 T cells from healthy, HHV-8 seropositive, HIV-1 negative adults controlling infection, with a portion of T cells producing 2-4 of these factors. These immunogenic regions of the viral proteins could be important in the immunopathogenesis of HHV-8 infection and the progression to Kaposi's sarcoma.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

PHENOTYPIC VARIATION IN EPSTEIN-BARR VIRUS (EBV)-IMMORTALIZED B CELL LINES: THE EFFECT OF CELLULAR FACTORS ON LYTIC REACTIVATION

**Authors and  
Affiliation:**

Michael L. Davies, Shushen Xu and David T. Rowe (Department of IDM, GSPH)

**Text:**

**Objectives:** B-Lymphoblastoid cell lines (LCLs) are widely used in studies on the mechanisms of cellular transformation, and as autologous targets in studies of cell-mediated immunity. They are generally treated as interchangeable, but LCLs have been found to vary widely in viral content and cellular phenotype, with unknown implications for their utility as a research tool. Our aims were

**A)** to characterize a range of LCLs for their EBV DNA content, EBV mRNA expression, and cellular properties such as surface marker expression, with the hope of identifying factors that could categorize LCLs into functional subtypes;

**B)** to study the levels of lytic EBV reactivation in LCLs, using them as a model for identifying cellular factors that cause a latently EBV-infected cell to switch to activation of the lytic EBV life cycle.

**Methods:** LCLs were created by infecting PBMCs *in vitro* with the B95-8 strain of EBV and allowing immortalized B cells to grow out, and were grown for at least 2 months before characterizing. Real-time PCR for EBV DNA was used to determine the number of viral genomes per cell; quantitative RT-PCR, with mRNA-specific primers (normalized to  $\beta_2$ -microglobulin), was used to determine viral gene expression. The percentage of cells experiencing viral reactivation was measured by intracellular flow cytometry for the lytic EBV proteins ZEBRA and gp110. Cellular phenotypes were determined by flow cytometry for cell surface markers, as well as monitoring their morphology and growth rate. We isolated RNA from LCLs, synthesized cDNA, and used Illumina BeadChip microarrays to identify cellular genes which correlated with high or low levels of lytic reactivation; TaqMan Low-Density PCR Arrays were used to confirm these correlations.

**Results:** LCLs ranged from <20 to >1,000 EBV genome copies per cell, a range which was similar in cell lines derived from juvenile and adult donors. This property was stable over time and was not found to correlate with the cell lines' growth rate, morphology, or any phenotypic cell-surface markers such as HLA Class I or II, surface antibody, activation markers, or memory or naïve B cell lineage markers. The amount of EBV DNA in a cell line was found to correlate with the frequency of lytic EBV reactivation in that cell line, but not with the expression of any latent gene products. LCLs could be brought down from >1,000 to <100 EBV genomes/cell by the addition of the antiviral drug acyclovir; this had no detectable effect on other aspects of the LCLs' phenotype, and upon removal of the drug the LCLs quickly returned to their high-lytic reactivation status. This indicates that the propensity for viral reactivation is likely a result of cellular factors. Microarrays suggested several cellular genes and families of genes for which the quantity of mRNA correlated with an LCL's status as high- or low-viral reactivation. Follow-up RT-PCR assays on a larger sample of LCLs confirmed this relationship for certain genes, including those for transcription factors *KLF2*, *ATF5*, *EBF1*, and *GFII*; chemokines *CCL3* and *CCL4L1*; chemokine receptor *CCR10*; and G protein *GNA15*.

**Implications:** By identifying an LCL's propensity for EBV lytic reactivation as a stable property which varies widely across cell lines, we can use LCLs to identify cellular factors which influence the lytic switch. Further studies on the roles of these potential factors (especially *KLF2*, which plays a role in inducing quiescence and inhibiting activation of lymphocytes) will involve siRNA knockdown and overexpression, to see if modulating cellular factors can lead to modulation of viral reactivation.

Also, although panels of antibodies for B-cell surface markers did not associate an LCL's cellular phenotype with its propensity for viral reactivation, we learned new details on the range of LCL surface-marker phenotypes. Further studies into how an LCL changes over time in EBV content – both lytic virions and latent episomes – would also be informative.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**GENETIC DETERMINANTS ASSOCIATED WITH DYSLIPIDEMIA IN HIV POSITIVE PATIENTS  
RECEIVING ANTIRETROVIRAL THERAPY**

**Authors and  
Affiliation:**

**Matthew Nicholaou, Jeremy Martinson, Lawrence Kingsley**

University of Pittsburgh Graduate School of Public Health, Department of Infectious Diseases and  
Microbiology

**Text:**

**Statement of Problem:** The use of highly active anti-retroviral therapy (HAART) in the treatment of HIV infection has resulted in a number of observed metabolic disorders. Some patients present an abnormal lipid profile that resembles individuals at high risk of cardiovascular disease in the general population.

**Methods:** We used a cross-sectional study design to analyze participants in the Multicenter Aids Cohort Study (MACS) in the year 2005. This cohort study gives us detailed lipid profiles from 2,534 HIV seropositive (1181) and seronegative (1353) individuals. We have defined a patient as having the dyslipidemia phenotype if they have a LDL level in the top quartile of the population and HDL level in the bottom quartile of the population. Cases and controls for each phenotype are independent. Genomic DNA has been extracted from 1,930 PBMC cell pellets using the Qiagen DNeasy Blood and Tissue Kit. Genotype data for 4 SNPs in these individuals was obtained using PCR based Fluorescence Polarization (FP).

**Data Analysis/Results:** We have genotyped approximately 640 of these patients for 4 SNPs across 4 genes known to influence lipid metabolism; PLTP (1b+26), ABAC1 (32b+30), LPL (S447X), and SR-B1 (A350A). We have estimated, with our current resources, that we can determine approximately 1,000 genotypes per week. For the study of a multi-factorial disease that involves hundreds of genes and thousands of SNPs within these genes; this approach is unrealistic. Given this fact, we intend to change our technique to a higher throughput assay. Our lab will utilize Illumina's GoldenGate assay to achieve the high throughput genotyping needed in this study. We have selected 100+ genes associated with altering lipid metabolism and will screen these genes for 1,536 previously identified lipid relevant SNPs. The distributions of these SNPs will be compared across cases and controls for each disease phenotype. Haplotypes of linked SNPs will be constructed and used for addition associations between disease phenotypes.

**Implications:** By identifying the genetic determinants linked to dyslipidemia in our population, we hope to describe the contribution host genetics has on these observed lipid abnormalities. In addition, the identification of these polymorphisms could lead to a genetic screen that would allow for the prediction of dyslipidemia in our population.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract****Title:**

**Nabanita Biswas** and Tianyi Wang

**Authors and  
Affiliation:**

Department of Infectious Diseases & Microbiology, University of Pittsburgh, Graduate School of Public Health, 130 Desoto Street, Pittsburgh, PA 15261, USA.

**Text:**

**Background:** The innate immune system is the first line of host defense against invading pathogens. Type 1 interferon plays an important role in innate immunity by promoting the expression of specific cellular proteins, some of which are involved in the inhibition of virus replication and particle formation. Among different interferon inducible genes, ADAR1 (adenosine deaminase acting on RNA 1) catalyzes hydrolytic deamination of adenosine to inosine in complete or partially double-stranded RNA, which are common intermediate products found during viral infections.

**Objective:** Results from a few published reports suggest that ADAR1 potentially regulates Hepatitis C virus (HCV) and Influenza A virus infections. However, the direct effect of ADAR1 on virus infection remains elusive. Here our objective is to determine the role of ADAR1 in anti HIV-1 immunity.

**Result and conclusion:** We have shown by p24 ELISA and TZM assay that ADAR1 inhibits the production of infectious HIV-1 in 293T cells. An 8-fold decreased in the viral particle formation was observed when the highest concentration of ADAR1 was used. The same phenomenon was observed in Hela cells and Jurkat T cells. By using different ADAR1 domain mutant it was also observed that catalytic domain of ADAR1 and the double-stranded RNA-binding domain appear to be critical in restricting the production of infectious HIV-1 particles. So, we concluded that ADAR1 restricts HIV replication and infectivity.

**Implication:** Evaluating the RNA editing effects of ADAR1 during HIV infection and understanding the mechanism(s) by which viral production is inhibited may provide further insight into the host cell-pathogen interaction and reveal an important mediator of anti-viral innate immunity.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

SELECTIVE ACTIVATION OF THE SRC-FAMILY KINASES HCK AND LYN IS A GENERAL PROPERTY OF NEF PROTEINS FROM MAJOR HIV-1 SUBTYPES

**Authors and  
Affiliation:**

**Purushottam S. Narute**<sup>1</sup> and Thomas E. Smithgall<sup>2</sup>

<sup>1</sup>Infectious Disease and Microbiology, Graduate School of Public Health and <sup>2</sup>Microbiology and Molecular Genetics, School of Medicine, University of Pittsburgh, Pittsburgh PA 15261

**Text:**

Background: HIV Nef is a small myristoylated protein expressed early in the HIV-1 life cycle and essential for high-titer viral replication and AIDS progression. Nef lacks enzymatic activity, and exerts its effects by interacting with many host cell molecules. In particular, Nef interacts with the SH3 domain of Hck and other Src-family protein-tyrosine kinases (SFks) through its highly conserved proline-rich (PxxPxR) motif and hydrophobic pocket. Nef alone is sufficient to produce AIDS-like symptoms in transgenic mice and this effect is dependent in part upon the PxxP motif and Hck. Previous studies from our laboratory have shown that Nef proteins derived from laboratory strains of HIV-1 (e.g., Nef-SF2) induce constitutive activation of Hck and Lyn in vitro and in several cell-based systems (yeast, fibroblasts, and myeloid cells).

Objective: To evaluate the ability of Nef alleles representative of all major HIV-1 subtypes to activate SFks expressed in HIV-1 target cells.

Methods: Nef proteins from most major HIV-1 clades were purified from bacterial using His-Ni-NTA affinity chromatography. The binding of HIV-1 Nef to Hck SH3 domain was evaluated by pull-down assay. Nef-mediated SFk activation was evaluated in our well-established yeast-based model system. Briefly, down-regulated forms of SFks and each HIV-1 Nef subtype were co-expressed in yeast and kinase activation was evaluated by anti-phosphotyrosine immunoblots of yeast cell lysates.

Results: All primary Nef proteins tested showed strong binding to the Hck SH3 domain. Similar to our previous reports with Nef-SF2, all Nef alleles selectively activated Hck and Lyn, but have no effect on other SFks expressed in hematopoietic cells (Fgr and Lck).

Conclusion: Our results indicate that selective activation of Hck and Lyn among SFks expressed in HIV-1 target cells is a general property of HIV-1 Nef. Selective inhibitors of Nef:SFk signaling may block Nef-dependent functions in HIV-infected cells independent of the HIV-1 subtype.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract****Title:****Authors and  
Affiliation:****Text:****DEVELOPMENT OF A NOVEL VACCINE STRATEGY AGAINST RIFT VALLEY FEVER VIRUS**

**Bhardwaj, Nitin**<sup>1,2</sup> and Ross, Ted.M.<sup>2</sup>

1 Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, 2 Center for Vaccine Research University of Pittsburgh School of Medicine, Pittsburgh PA USA 15261

**Objectives:** Rift Valley Fever virus (RVFV) is an arthropod-borne Phlebovirus (family *Bunyaviridae*) associated with abortion storms, neonatal mortality in livestock and hemorrhagic fever or fatal encephalitis in a proportion of infected humans. Although, the inactivated RVFV vaccines have been used in livestock, there is no licensed vaccine available to protect the human population. Therefore, there is an urgent need for developing safe and effective vaccine that rapidly elicits protective immunity against RVFV infection.

**Methods:** To address this, DNA plasmids expressing RVFV Gn glycoprotein in conjunction with three copies of molecular adjuvant C3d were constructed and analyzed for its ability to act as a potent vaccine candidate against RVFV in mice. An experimental live-attenuated vaccine (MP-12) was used as a benchmark for comparison.

**Results:** The DNA plasmids successfully expressed RVFV glycoprotein Gn in conjunction with C3d and elicited anti-RVFV immune response after three immunizations as determined by anti-RVFV ELISA and neutralizing antibody response. Interestingly, our DNA plasmid derived vaccine elicited neutralizing antibody titers comparable to the MP-12 vaccine.

**Discussion:** DNA vaccines were constructed against RVFV and further compared with experimental live-attenuated MP-12 vaccine in mice. In these studies, the DNA plasmid constructs expressed successfully in cell culture and was able to induce anti-RVFV antibody response when administered to mice. Furthermore, sera from vaccinated mice neutralized virus.

**Public Health Implications:** Rift Valley Fever virus represents a significant threat to human health and there is a pressing need for the development of improved vaccines against this pathogen. This is a promising approach with DNA derived vaccines which will not only directly assess their potential as a vaccine candidate, but will also significantly enhance our general understanding of anti-RVFV immunity.

**CATEGORY OF EMPHASIS**

(check all that apply)

 Bioscience Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**INDUCTION OF THE ANTI HIV-1 POLYFUNCTIONAL T CELLS IN THE MUCOSAL COMPARTMENTS USING A PRIME BOOST APPROACH USING RECOMBINANT CLOSTRIDIUM PERFRINGENS AND VIRUS LIKE PARTICLES.**

**Authors and  
Affiliation:**

**Poonam, P\***, Helmus R\*, Chen, Y\*, McBurney, SP#, Ross, TM#, and Gupta, P\*

\*Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh;

#Department of Medicine, Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

**Text:**

**Background:** A mucosal site such as gastrointestinal tract is a major target for early HIV-1 infection. Strong immune responses against HIV-1 at these sites may protect early infection. Recombinant *Clostridium perfringens* has been shown to deliver SIV/HIV-1 proteins in large quantities to the terminal ileum in order to induce strong mucosal immune responses. Virus like particles (VLPs) has been shown to induce strong mucosal responses when delivered intranasally. Therefore a mucosal immunization strategy using *C. perfringens* and VLPs should be able to induce potent immune responses against HIV-1 in the gut and other mucosal tissues. **Objective:** To induce strong mucosal immune responses against HIV -1 using prime boost approach using recombinant *C. perfringens* expressing HIV-1 proteins and HIV-1 VLPs. **Methods:** Mice were immunized either orally with a recombinant noncytotoxic (cytopathic gene deleted) *C. perfringens* expressing HIV-1 p55 protein (CP-p55) or intranasally with HIV-1 VLPs in prime boost approaches along with optimized adjuvants. T cell mediated immune response in the spleen, mesenteric lymph nodes (MLN), peyer's patches (PPs) and lamina propria of the immunized mice were determined using ELISPOT by using CD3, CD4 or CD8 cells. Intracellular cytokine staining was performed to detect HIV-1 specific cytokines. HIV-1-specific IgA and IgG antibodies were also measured in mucosal and serum samples. **Results:** HIV-1 specific mucosal immune responses in both the effector and inductive sites of the gut were significantly higher in mice immunized using recombinant CP-p55 and HIV-1 VLPs in heterologous prime-boost approaches compared to mice immunized with either CP-p55 or HIV-1 VLPs alone. These groups also had higher proportions of HIV-1 specific polyfunctional T cells when compared to the mice immunized with either CP-p55 or HIV-1 VLPs alone. However no significant HIV-1 specific humoral immune responses were detected in the mucosal or systemic compartments of the immunized mice. **Conclusions:** Mucosal immunization with a combination of CP-p55 and HIV-1 VLPs led to a strong HIV-1 specific cellular immune response in mucosal immune compartments. Moreover this approach was successfully able to prime gut mucosal immune responses against HIV-1, which will be essential to counter HIV-1 infection at the gut in the initial stages of HIV-1 infection.

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**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract Title:**

**TO COMPARE THE BRONCHODILATORY (BD) EFFECTS OF SHORT ACTING BETA AGONISTS (SABA) – SALBUTAMOL AND SHORT ACTING ANTICHOLINERGIC AGENTS (SAAC) – IPRATROPIUM AMONG PATIENTS WITH VARYING SEVERITY OF ASTHMA AND COPD.**

**Authors and Affiliation:**

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**Text:**

**OBJECTIVES:** Variable bronchodilator responses to SABA and SAAC agents are seen in subjects with Asthma and COPD. The bronchodilator response to SABA wanes with age in subjects with asthma, whereas the response with SAAC remains unaltered and increases after the age of 40 years<sup>1</sup>. To test this hypothesis, the upper airway and lower airway bronchodilatory responses of Salbutamol and Ipratropium, both delivered by pressurized metered dose inhalers (pMDI) among Asthmatic and COPD patients were compared.

**METHODS:** A total of 40 newly diagnosed or those with preexisting Asthma /COPD subjects (current medications were stopped for a minimum of 12 hours prior to testing) performed both pre and post Salbutamol Spirometry, Impulse oscillometry (IOS) and Body plethysmography on Day1. The same was repeated with Ipratropium 24 hours later on Day2. Airway responses (upper and lower airway BD effects) and the reversibility in Forced expiratory volume in the first second (FEV<sub>1</sub>), improvement in Forced expiratory flow<sub>25-75</sub> (FEF<sub>25-75%</sub> is the average expired flow over the middle half of the Forced Vital Capacity manoeuvre - regarded as a more sensitive measure of small airways narrowing than FEV<sub>1</sub>) were noted on Spirometry. Residual volume and Total lung capacity (pre and post) were recorded in Body plethysmography measurements. Impedance at both lower and high frequencies was measured in Impulse Oscillometry System.

**RESULTS:** 20 among the 40 subjects were asthmatics and the rest were subjects with Chronic Obstructive Pulmonary disorder. 19 COPD subjects were more than 40 years of age. 14 Asthmatics were more than 40 years at the time of study. The mean reversibility difference in FEF<sub>25-75</sub> between Salbutamol and Ipratropium amongst Asthmatics was -18.48 % (95% CI: - 26.46, -10.50) which was statistically significant (p<0.0001). The mean reversibility difference in FEF<sub>25-75</sub> between Salbutamol and Ipratropium amongst COPD subjects was -16.52 % (95% CI: - 23.66, - 9.37) which was statistically significant (p<0.0001). No significant difference in FEV<sub>1</sub> between Salbutamol and Ipratropium amongst Asthmatics was observed, -2.14 % (95% CI: -8.68, 4.39) (p=0.49). The mean reversibility difference in FEV<sub>1</sub> between Salbutamol and Ipratropium amongst COPD subjects was - 3.82 % (95% CI: 0.69, -8.34) which trended towards statistical significance (p=0.09).

**IMPLICATIONS:** The disability in elderly Asthmatics and COPD patients is due to small airway narrowing and dysfunction. Smaller airways (measured by FEF<sub>25-75</sub>) respond better to Ipratropium amongst both Asthmatics and COPD subjects. Reversibility testing with Salbutamol and Ipratropium should routinely incorporate testing for smaller airway functions. Recommendations and policy action plan regarding the usage of bronchodilators among Asthmatics and COPD should advocate usage of Ipratropium or continued therapy with both drugs in older patients.

References:

1. **MI Ullah, GB Newman and KB Saunders.** Influence of age on response to ipratropium and salbutamol in asthma. Thorax, Vol 36, 523-529, 1981.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

A RETROSPECTIVE CHART REVIEW OF FEBRILE INFANTS WITHOUT MENINGITIS TO  
 ACCURATELY DETERMINE NORMAL COMPONENTS OF CEREBROSPINAL FLUID

**Authors and  
Affiliation:**

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 Pittsburgh Medical Center. Pittsburgh, PA

**Text:**

**Description of the Problem:** Lumbar puncture is a common procedure in the evaluation of febrile infants in hospitals across the United States. Although this procedure carries some risks, it is the only effective way to quickly and accurately diagnose bacterial and viral meningitis in infants. Because treatment initiation is critical to outcome, there is typically not enough time to wait for cerebrospinal fluid (CSF) bacterial and viral cultures. Therefore, the CSF is analyzed for surrogate markers of infection including white blood cell (WBC) count, glucose, and protein. Although WBC count, protein, and glucose level perturbations have been previously described in infants with bacterial and viral meningitis, the literature fails to accurately describe these indices in “normal” infants. Therefore, defining these reference values will help physicians more accurately diagnose and guide treatment of suspected meningitis in infants.

**Approach Taken:** A retrospective chart review will be conducted on three cohorts of infants that presented with fever at the Children’s Hospital of the University of Pittsburgh Medical Center. The analysis of data from three different time periods within the past twenty years will ensure that diagnostic criteria remain constant and are not significantly skewed due to advances in diagnostic sensitivity. Approximately 1500 febrile infants less than eight weeks of age who successfully underwent lumbar punctures, had negative viral and bacterial cultures (CSF, blood, and urine), were not born prematurely, and had no history of seizures will be included in this study. Additionally, infants will be excluded from the study if their CSF RBC counts are indicative of a traumatic lumbar puncture (>500 RBC/μL).

**Data Analysis:** Following chart selection, the chosen cases will be stratified by age in weeks and subjected to statistical analysis in order to determine if the means, medians, and confidence intervals vary significantly between febrile infants of different ages. These tests will be conducted to evaluate the CSF WBC counts, protein, and glucose concentrations for each population.

**Implications:** These reference values will serve as a valuable diagnostic tool for physicians treating febrile infants with suspected meningitis in the absence of CSF culture.

**CATEGORY OF EMPHASIS**

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Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

ONE WORLD...ONE HEALTH

**Authors and  
Affiliation:**

Sheri Hathaway BSN

**Text:**

**Objectives:** To inform residents of Greene County, Pennsylvania about potential risks associated with consuming game (white-tailed deer) infected with Epizootic Hemorrhagic Disease (EHD).

**Approach Taken:** Editorial article in Observer Reporter in Washington, Pa.

**Results:** In the fall of 2007 EHD reached epidemic proportions in the white-tailed deer population in SW Pennsylvania, especially Greene County. Experts from the Pennsylvania Game Commission (PGC) gave a presentation on EHD to local hunters. A bias favoring the PGC and revenue generated by the sale of hunting licenses exists by not having the public health sector present at the meeting to detail epizootic infections and emerging diseases. Public Health must be a multi-disciplinary effort to ensure the health of all life on our planet. One World...One Health.

**Conclusion:** Many articles were published thereafter related to EHD in Greene County as a result of public inquiry.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**Tight Junction Proteins Claudin-1 and Occludin Control Hepatitis C Virus Entry and are Downregulated during Infection to Prevent Superinfection**

**Authors and  
Affiliation:**

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**Text:**

**Background:** The tight junction (TJ) protein, Claudin-1 (CLDN1), was identified recently as a key factor for Hepatitis C virus (HCV) entry. TJs are dynamic cellular structure where frequent exchange of TJ components and their intracellular pools occurs. It is currently unclear whether HCV enters liver cells through the TJ and how this process happens.

**Objective:** To investigate the significance of the tight-junctional CLDN1 in viral entry.

**Result and conclusion:** We show that another TJ protein, occludin, is also required for HCV entry. Mutational study of CLDN1 revealed that its tight junctional distribution plays an important role in mediating viral entry. Together, these data support the model that HCV enters live cells from the TJ. Interestingly, HCV infection of Huh-7 hepatoma cells downregulated the expression of CLDN1 and occludin, preventing superinfection.

**Implication:** The altered TJ protein expression may contribute to the morphological and functional changes observed in HCV diseased livers.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract  
Title:**

**ASSOCIATION BETWEEN DECREASED CXCL12 AND CCL25 EXPRESSION AND INCREASED APOPTOSIS IN LYMPHOID TISSUES OF CYNOMOLGUS MACAQUE DURING SIV INFECTION**

**Authors and  
Affiliation:**

**Shulin Qin<sup>1</sup>, Yongjun Sui<sup>1,2</sup>, Michael A. Murphy-Corb<sup>1</sup> and Todd A. Reinhart<sup>1</sup>**

**Text:**

**<sup>1</sup>Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh; <sup>2</sup>Current address: Vaccine Branch, Molecular Immunogenetics and Vaccine Research Section, National Cancer Institute, 10 Center Drive, Bethesda, MD 20892-1578**

Chemokines are small chemoattractant cytokines that likely play multiple roles in HIV/SIV pathogenesis. To examine potential associations between chemokine expression levels and apoptosis of cells in lymphoid tissues during SIV infection, we measured chemokine and cytokine mRNA levels using real-time RT-PCR in multiple lymphoid tissues compartments from uninfected and SIV-infected cynomolgus macaques and found that CXCL12 and CCL25 mRNAs in SIV-infected lymphoid tissues were decrease. Immunohistochemical detection of activated caspase-3 and Ki67 as markers for apoptosis and proliferation of cells, respectively, revealed increased numbers of proliferating and apoptotic cells in lymphoid tissues during acute SIV infection and AIDS. Correlation analyses revealed that CXCL12 and CCL25 mRNA levels in lymphoid tissues were negatively correlated with the numbers of proliferating and apoptotic cells and the numbers of these kinds of cells in lymphoid tissues were positively correlated with local SIV viral loads, IFN- $\gamma$  and pro-inflammatory chemokines expression levels. In vitro analyses revealed that CXCL12 and CCL25 were capable of reducing apoptosis induced by SIV infection. These findings suggest that increased apoptosis in lymphoid tissues due to reduced levels of anti-apoptotic chemokines might be a mechanism contributing to loss of immune function following SIV infection.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention

**ABSTRACT FORM**  
**Annual IDM Research Day**  
**September 17, 2008**

**Abstract Title:**

Abstract Title: MECHANISTIC STUDIES ON A CD8 SUPPRESSIVE FACTOR THAT INHIBITS HIV-1 REPLICATION

**Authors and Affiliation:**

**Varsha Shridhar**, B.Tech\*, Ashwin Tumne, PhD<sup>#</sup>, Yue Chen, PhD<sup>#</sup> and Phalguni Gupta, PhD<sup>#</sup>

**Text:**

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CATEGORY OF EMPHASIS: Bioscience

Abstract

Introduction: CD8+ cells from HIV infected individuals can inhibit viral replication in acutely infected CD4+ cells in a non-cytolytic manner by suppressing transcription from the HIV promoter, the long terminal repeat (LTR). The mechanism of non-cytolytic anti-viral action is strongest when there is cell to cell contact between the CD8+ and the CD4+ cells, though CD8 culture supernatant has also been found to significantly (>80%) suppress HIV transcription.

In our efforts to study the mechanism of suppression and identify the factor responsible, we recently detected a membrane-bound HIV-1 suppressing activity secreted as 30-100 nm sized exosomes from transformed CD8+ T cells. The exosomes were found to be a good model for these studies.

Objective: To elucidate the process by which the suppressive factor produced by CD8 T cells, and present on CD8 exosomes, mediates its effects on HIV LTR to inhibit transcription.

Methods: The interaction between the exosomes and the target cells was studied by confocal microscopy, using Cy5 (red) labeled exosomes and PKH67 (green) labeled target cells. Cell lines knocked out for individual signaling molecules were used to identify intracellular signaling molecules. To localize the region on the LTR crucial for suppression of transcription, deletion mutant analyses of the LTR were performed.

Results and Discussion: Confocal microscopy shows that the interaction of exosomes with the cells is restricted to the surface and occurs rapidly (within 10 minutes of addition). However, significant suppression of LTR driven transcription is observed only after 12-16 hours. This suggests the possible production of an intermediate protein. Studies using the knock-out cell lines suggest the involvement of STAT1 in this process. Experiments using deletion mutants of the HIV LTR suggest that the region necessary for suppression is the TAR (trans-activation responsive) region. This is very intriguing because the TAR region is not only crucial for transcription of the HIV genes, but is also one of the conserved regions in the HIV genome. This could explain why the suppressive factor is effective regardless of the subtype or the strain of the virus. Thus, elucidation of the molecular mechanisms of suppressive factor action has great significance both in the fields of viral immunology as well as therapy.

**CATEGORY OF EMPHASIS**

(check all that apply)

Bioscience

Education/Prevention