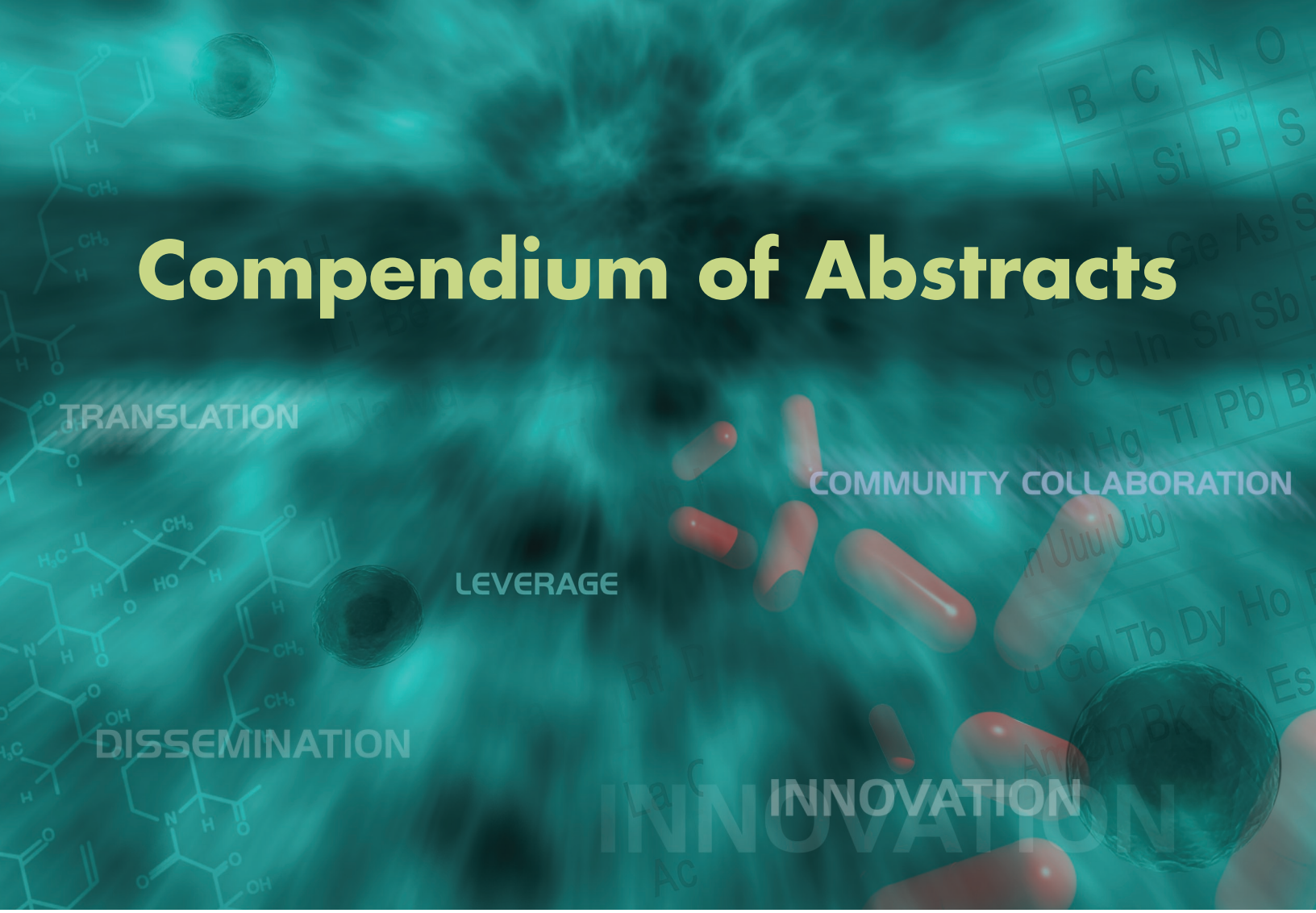

Compendium of Abstracts

21st HIV/AIDS Biennial Investigators' Meeting
7th Conference on AIDS Research in California

California HIV/AIDS Research: Accelerating Discovery

February 24, 2006 • San Mateo, California

Presented by the Universitywide AIDS Research Program
University of California, Office of the President

Compendium of Abstracts **2006**

21st HIV/AIDS Biennial Investigators' Meeting
7th Conference on AIDS Research in California

California HIV/AIDS Research: Accelerating Discovery

February 24, 2006 • San Mateo, California

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GRANT CODE KEY

Each abstract in this compendium has an associated grant code which indicates the award type (mechanism) and the grant cycle (year awarded). An example is:

ID04-LA-018

The first one or two letters indicate the mechanism, while the next two numbers indicate the grant cycle. So for the above example this was an IDEA from the 2004 grant cycle. *Note that this compendium does not contain abstracts from the 2006 grant cycle.* The next designation is the institution, in this case UCLA, and the last numbers are a unique identifier used by UARP.

There are 6 mechanisms that are part of the regular Call for Applications:

ID	IDEA (Innovative, Developmental, Exploratory Award)
CR	Community Collaborative Research Award
F	Postdoctoral Research Fellowship
CF	Clinical Research Fellowship
TF	Biomedical Translational Research Fellowship (none in this compendium as the first was awarded in the 2006 grant cycle).
D	Dissertation Award

You will see several other grant codes in this compendium from awards that come from RFAs, RFQs and the like that were not part of the regular call for applications. Examples include:

CH	California Collaborative HIV/AIDS Research Centers
HP	Health Policy Research Initiative
CM	California Mexico AIDS Initiative
CC	California AIDS Research Centers (replaced by CH in 2005)

California HIV/AIDS Research: Accelerating Discovery

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7:00 am	**Registration & Continental Breakfast**	*Golden Gate Room*
8:30–9:45 am	**Breakout Sessions A**	*Breakout rooms*
	Anti-HIV Therapeutic Strategies Opportunistic Infections, Malignancies, and Model Systems HIV/AIDS Policy and Health Care Financing HIV and African Americans: Social and Behavioral Risk Factors	
9:45–10:00 am	**Break**	
10:00–11:30 am	**Breakout Sessions B**	*Breakout rooms*
	Innate Immunity and Host Factors Opportunistic Infections and Related Topics Psychosocial and Mental Health Issues & HIV/AIDS Services and Care HIV and Latinos: Social and Behavioral Risk Factors	
11:30 am–1:00 pm	**Luncheon**	*Golden Gate Room*
	Update on UARP Strategic Planning Process: George Lemp, Director, Universitywide AIDS Research Program Gregory Melcher, Vice-Chair, Universitywide Task Force on AIDS	
1:00–2:30 pm	**Breakout Sessions C**	*Breakout rooms*
	Latency and Viral Mechanisms Vaccine Development and Immunology—I Health Services Research and Quality of Care HIV and Communication: Dissemination, Technology Transfer and Translation HIV and Men: Social and Behavioral Risk Factors	
2:30–2:45 pm	**Break**	
2:45–4:30 pm	**Breakout Sessions D**	*Breakout rooms*
	HIV Clinical Research Topics and NeuroAIDS Vaccine Development and Immunology—II HIV and Youth: Social and Behavioral Risk Factors HIV and IDU/Substance Use: Social and Behavioral Risk Factors	

Section 1: Biomedical Sciences

Anti-HIV Therapeutic Strategies

Opportunistic Infections, Malignancies, and Model Systems

Innate Immunity and Host Factors

Opportunistic Infections & Related Topics

Latency and Viral Mechanisms

Vaccine Development & Immunology—I

HIV Clinical Research Topics and NeuroAIDS

Vaccine Development & Immunology—II

Compendium Only

Section 2: Health Services, Policy & HIV/AIDS Treatment and Care

HIV/AIDS Policy and Health Care Financing Research

Psychosocial and Mental Health Issues & HIV/AIDS Care and Treatment

Health Services Research and Quality of HIV/AIDS Care

Compendium Only

Section 3: HIV and Prevention

HIV and African Americans: Social and Behavioral Risk Factors

HIV and Men: Social and Behavioral Risk Factors

HIV and Youth: Social and Behavioral Risk Factors

HIV and IDU/Substance Use: Social and Behavioral Risk Factors

Biomedical Sciences 1

Anti-HIV Therapeutic Strategies

Discovery of a Novel Small Molecule HIV-1 Integrase Inhibitor Binding-Site

Presenter: Nouri Neamati, University of Southern California, Los Angeles

Collaborators: Laith Q. Al-Mawsawi, Valery Fikkert, Raveendra Dayam, Myriam Witvrouw, Terrence R. Burke Jr., Christoph Borchers

Principal Investigator: Nouri Neamati

UARP Award Number: ID04-USC-049

Background: Structural information detailing the association between IN and inhibitors under development is of enormous therapeutic importance. Knowledge of key amino acid residues involved in the binding of potential drug, and therefore which residues are likely to mutate under therapeutic pressure, would inevitably help researchers stay one step ahead of drug resistant viral strains. Herein, we report the identification of a unique HIV-1 Integrase (IN) inhibitor binding site using photoaffinity labeling and mass spectrometric (MS) analysis.

Methods: We chemically incorporated a photo-activatable benzophenone moiety into a series of coumarin-containing IN inhibitors. A representative of this series was covalently photo-crosslinked with the IN core domain and subjected to HPLC purification. Fractions were subsequently analyzed using MALDI-MS and ESI-MS to identify photo-crosslinked products.

Results: We identified a single binding site for an inhibitor located within the tryptic peptide 128AACW-WAGIK136. Site-directed mutagenesis followed by in vitro inhibition assays resulted in the identification of two specific amino acid residues, C130 and W132, in which substitutions resulted in a marked resistance to the IN inhibitors. Docking studies suggested a specific disruption in functional oligomeric IN complex formation.

Conclusions: The combined approach of photo-affinity labeling/MS analysis with site directed mutagenesis/molecular modeling is a powerful approach for elucidating inhibitor binding-sites of proteins at the atomic level. This approach is especially important for the study of proteins that are not amenable to traditional x-ray crystallography and NMR techniques. This type of structural information can help illuminate processes of inhibitor resistance, and thereby facilitate the design of more potent second-generation inhibitors.

Anti-HIV Therapeutic Strategies

A Biosensor for Detection of HIV Fusion Inhibitors

Presenter: Miriam Gochin, Touro University

Collaborators: Yanxia Hou, Vino P. Madhavan

Principal Investigator: Miriam Gochin

UARP Award Number: ID05-TOURO-041

Background: Human Immunodeficiency Virus Type 1 (HIV-1) envelope protein gp41 regulates viral – host cell fusion, and is an important target in the development of entry inhibitors to control HIV infection. Currently the peptide Enfuvirtide® is the only approved fusion inhibitor. Small molecule drugs that inhibit fusion could be an effective addition to the anti-HIV therapeutic arsenal. They are typically designed to disrupt the formation of the six-helix bundle conformation of fusion-competent gp41. There are large repositories of compounds in national laboratories and pharmaceutical companies that could be screened for compounds active against HIV-1 fusion, provided that a cost-effective screening procedure was available. We describe methodology to create a solid-state biosensor that will be able to rapidly and efficiently detect compounds with potential anti-fusion properties.

Methods: Prototype non-peptide fusion drugs have mostly been targeted to a hydrophobic pocket known to exist in the trimeric coiled coil core domain (HR1) of gp41. The HR1 domain is not stable when excised from full length gp41 nor exposed in solution in intact gp41. To make it available as a target in a biochemical assay, we are developing a self-assembled monolayer (SAM) of a segment of gp41 HR1 anchored on a gold-plated quartz crystal. Integrity of the trimeric coiled coil structure will be preserved through the use of a transition metal ion coordinated to a metal-ligating N-terminal bipyridyl group. Three ligands binding tightly to the metal and hydrophobic peptide-peptide contacts should ensure the trimeric folded form of the HR1 domain. We are testing the formation and stability of the monolayer using an electrochemical quartz crystal nanobalance (EQCN) to measure mass changes at the surface due to SAM formation, and electrochemical changes due to the redox properties of the metal ion. Confirmation will be obtained using scanning electron microscopy or atomic force microscopy. The sensitivity of the physicochemical properties to the binding of inhibitors to the SAM will be tested, initially with peptides known to bind tightly to the HR1 domain. Using a flow cell, our ability to regenerate the sensor for repeated use will be tested. The product envisioned is a nanoscale sensor that could detect the presence of a binding molecule in 5-10μL of solution upon contact. The correlation between binding and fusion inhibition will be tested using a cell-based fusion assay.

Expected results: A 26-residue peptide has been designed for the initial SAM formation. It contains an N-terminal 2,2'-bipyridyl group, connected to the linker sequence GQAV, followed by 21 residues of wild-type gp41 HR1 encompassing the hydrophobic pocket, and a C-terminal D-cysteine residue for thiol adsorption on gold, while maintaining vertical positioning of the coiled coil with respect to the surface. The peptide will be ligated to metal ion CoII, NiII, FeII or RuII prior to SAM formation. We expect to observe a strong signal associated with peptide attachment using the EQCN, and a cyclic voltammogram associated with the metal. Signals should reflect stability and consistency of the monolayer. An 18-residue peptide modeled after the gp41 HR2 domain has been developed and shown to bind with 2μM affinity to the coiled coil. Exposure of the SAM to a solution of this peptide should result in changes in mass, CV or electron transfer rate as a result of peptide binding to the SAM. Changes will be calibrated in order to establish the range and sensitivity of the method for the detection of small molecule binders. We have a cell-cell fusion assay and access to viral infectivity assays for correlation of observed binding to anti-fusion properties.

Anti-HIV Therapeutic Strategies

Design, Synthesis, and Evaluation of New HIV-1 DIS Binders

Presenter: Victor Tam, University of California, San Diego

Principal Investigator: Victor Tam

UARP Award Number: D04-SD-409

Background & Significance: With over 40 million people living with HIV at the end of 2004(as reported by the World Health Organization), there is an urgent need to address this global epidemic. Current treatments that include protease inhibitors and reverse-transcriptase inhibitors have slowly become ineffective as HIV mutates into new resistant strains. A new strategy to aid in this fight may involve targeting specific regulatory steps in the HIV viral life cycle with small molecules.

Our efforts in the past year have been focused on an underdeveloped target -- the HIV-1 Dimerization Initiation Site (DIS). This RNA site stabilizes the dimeric nature of retroviral genomes and is involved with reverse transcription and viral encapsidation. Advances with other HIV RNA sites in the viral genome include the Rev Response Element (RRE) and Transactivating Response Element (TAR), which have both shown nanomolar affinity for small molecules, suggest the DIS can also be targeted in a similar fashion. In addition, the DIS is structurally similar to the prokaryotic A-site whose small molecule-RNA interactions have been thoroughly studied in the past decade.

We report our findings and work involving a strategy characterized by: a) a structure-based method for modeling new drug candidates, b) an efficient synthesis of various small molecules and analogs, and c) a fluorescence-based drug-binding assay.

Methods: Our work thus far has been to exploit the structural similarities between the HIV-1 DIS and the prokaryotic A-site to design aminoglycoside mimics that take advantage of the hydrogen-bonding interactions between ring I of paromomycin and RNA. The synthesis of these mimics has been based off of proven techniques heavily cited in literature. We have evaluated these mimics through several biophysical techniques to determine their binding affinity and interactions with a DIS RNA construct. Additionally, we have been developing a fluorescence-based assay in quantifying small molecule binding to the DIS.

Results: Unpaired A280 in HIV-1 DIS may possibly be responsible for the high affinity of aminoglycosides to this RNA structure. To exploit possible hydrogen-bonding interactions with A280, we have synthesized mimics that couple various pyrimidines with the necessary 2-deoxystreptamine ring. The scheme designed has allowed for a quick synthesis of numerous analogs. Our mimics are currently being evaluated for RNA binding affinity and structural interactions.

Replacement of A1492 in the prokaryotic A-site with fluorescent nucleobase analog 2-aminopurine (2AP) has acted as an excellent assay for quantifying small molecule binding. Given the striking structural similarity between the DIS and A-site, we are currently developing a similar fluorescence-based assay for evaluating small molecule binding to this portion of the HIV genome by replacing A272 with 2AP. Several biochemical and biophysical experiments are being employed to determine the specificity and selectivity of this assay for both known RNA-binding aminoglycosides and newly designed scaffolds.

Anti-HIV Therapeutic Strategies

Development of an HIV-1 Integrase Inhibitor Assay

Presenter: Ingrid Bahner, Children's Hospital, Los Angeles

Collaborators: M. Kagoda, Nouri Neamati, D. B. Kohn

Principal Investigator: Ingrid Bahner

UARP Award Number: ID04-CHLA-001

The development of drugs that interfere with HIV-1 integration has been slow in part because of the lack of a cell-based and integration-specific high-throughput screening assay.

We are developing such an assay using a lentiviral vector carrying a marker gene, whose lack of expression will be the measure for abrogation of inhibition. Because the lentiviral vector can infect cells only in a single round, the assay can be made specific for integration by virtue of timing the addition of the putative integration inhibitory compound such that entry and reverse transcription will have occurred prior.

Using a lentiviral vector carrying the enhanced green fluorescent protein (eGFP) gene and using a luminescence based cell viability assay, we translated the measurement of drug activity and drug toxicity to a 96 well assay format. Using this assay system in a 96 well format, we determined the specificity of the assay three fold. First, we employed a positive control consisting of a lentiviral vector that can not integrate because it lacks integrase and carries mutations in the conserved dinucleotide sequence in the long terminal repeat. The expression of green fluorescence produced by cells transduced with such a non-integrating lentiviral (NIL) vector is due to the amount of transient expression in the absence of integration. We used this NIL vector to optimize our signal to noise ratio by determining the optimal read-out time that allows us to distinguish eGFP fluorescence produced from integrated DNA from transiently produced eGFP fluorescence.

The specificity of the assay was further verified using the non-nucleoside reverse transcriptase inhibitor Nevirapine. Nevirapine was added to the cells timed for reverse transcription and added to the cells timed for integration, which resulted in both the appropriate positive control signal as well as the appropriate negative control signal, respectively. Lastly, specificity of the assay was also verified using two known integrase inhibitors and determining their IC50 in this assay. We are currently in the process of further verifying the assay by measuring on a molecular level the amount of integration and correlating it with the measured eGFP expression.

Anti-HIV Therapeutic Strategies

Nucleotide-Guanidinoglycoside Conjugates as Anti-HIV Agents

Presenter: Yitzhak Tor, University of California, San Diego

Principal Investigator: Yitzhak Tor

UARP Award Number: ID05-SD-017

The rapid appearance of resistant HIV-variants, adverse effects on contemporary drugs, recent indications for HIV "superinfections" and disappointing results with experimental vaccines, all necessitate the continuous development of independent therapeutic strategies to combat HIV infection. The goals of the proposed project are to design, synthesize and evaluate novel nucleotide-guanidinoglycoside conjugates as potential anti HIV-1 agents.

The underlying hypothesis guiding the proposed strategy is that the anti HIV efficacy of nucleoside-based Reverse Transcriptase inhibitors (NRTIs) can be enhanced by covalently conjugating their corresponding monophosphates to guanidinoglycosides, a novel family of cellular uptake vehicles that also exhibit high affinity to viral RNA sequences. Two objectives are therefore concomitantly met: (1.) RT inhibitors are actively transported into the cell and then released in a semi-activated form, hence the necessary monophosphorylation step is circumvented, and (2.) essential regulatory events involving viral-specific protein–RNA interactions (e.g., Rev–RRE) are inhibited.

The unique fundamental as well as practical features offered by this approach include: (1.) two distinct stages in the lifecycle of the virus are targeted with one anti-HIV agent, and (2.) facilitated import of semi-activated NRTI's may enhance the therapeutic factor of clinically proven agents (since fewer metabolic activation steps are needed and the released negatively charged nucleotide may reside longer in the cell), and possibly revive NRTI's that have clinically never materialized due to lack of appropriate metabolic activation.

Anti-HIV Therapeutic Strategies

Development of Novel DNA Binding HIV-1 Integrase Inhibitors

Presenter: Clay Wang, University of Southern California, Los Angeles

Principal Investigator: Clay Wang

UARP Award Number: ID05-USC-055

Background: Nature continues to a rich source of compounds that can inhibit HIV infection or modulate specific molecular targets associated with viral entry or replication. In this pilot study, a biosynthetic mechanism based production system will be developed to create analogs of the depsipeptide echinomycin and triostin and evaluate the new chemical entities for biological activity against HIV-1 integrase. The subject of this project echinomycin is a member of the quinoxaline natural products characterized by its C2 symmetric cyclic depsipeptide structure. A general feature of quinoxaline natural products is the bicyclic chromophores that allow the quinoxaline antibiotics to bisintercalate into DNA. Members of the quinoxaline antibiotics differ by the nature of the peptide backbone and the functional groups found on the DNA intercalating quinoxaline chromophore. Interestingly two members of the quinoxaline antibiotics, quinoxapeptin A and luzopeptin A are known HIV reverse transcriptase inhibitors. Our interest in quinoxaline antibiotics as HIV integrase inhibitors stems from earlier reports with lexitropsin and pyrrole imidazole polyamides demonstrating that DNA binding small molecules are potent HIV-1 integrase inhibitors.

Methods: The innovative aspect of this project is that we will biosynthesize novel "nonnatural" natural products using a strain of *Escherichia coli (E. coli)* that that has been bioengineered to heterologously express echinomycin and triostin biosynthesis genes isolated from the original producing streptomyces. By targeted manipulation of the natural product biosynthetic pathways, analogs with new structural features not otherwise found in nature can be produced. Since the whole process is biosynthetic, production scale up can be achieved by increasing the size of the bacterial culture.

Preliminary Results: This grant was awarded November 2005 therefore the study is still in its infancy. In this abstract we will be presenting only our preliminary results. In our preliminary studies, we have developed strains of E. coli that harbor plasmids containing the biosynthetic gene for either triostin or echinomycin. A high-density fermentation system has also been developed to produce triostin and echinomycin in amounts necessary for NMR and LC/MS characterizations. We also found that the natural echinomycin is a HIV-1 integrase inhibitor at 10μM. In the next two years we plan to develop new HIV-1 integrase inhibitors by leveraging the biosynthetic system we have developed.

Conclusions: DNA binding molecules such as echinomycin can inhibit HIV-1 integrase and the platform is set to make analogs of these DNA binding molecules in a biosynthetic fashion.

Opportunistic Infections, Malignancies, and Model Systems

The Role of Chemokines in AIDS-associated Lymphoma

Presenter: Daniel Widney, University of California, Los Angeles

Collaborators: Daniel Widney, Elizabeth C. Breen, Dorina Gui, Jon Said, Roger Detels

Principal Investigator: Otoniel Martinez-Maza

UARP Award Number: ID04-LA-039

Background: The overall aim of this study is to define the role of chemokines in the pathogenesis of AIDS-associated non-Hodgkin's lymphoma, a B cell cancer that occurs commonly in HIV+ subjects. Chemokines (chemoattractant cytokines) are cytokines that direct the movement of immune cells. The specific aims of this project were to: 1) elucidate in vivo expression of chemokine receptors and related ligands in subjects who developed AIDS-lymphoma and 2) define in vivo roles for selected chemokines in AIDS-lymphoma tumor cell growth using a SCID mouse model for non-Hodgkin's lymphoma.

Methods: We have identified a number of chemokines, as well as other molecules, as being associated with AIDS-lymphoma. This study was done using archival plasma from the Multicenter AIDS Cohort Study (MACS) at UCLA. The MACS is a study of the natural history of AIDS that was initiated more than 20 years ago. Subjects in the MACS are seen at two study visits per year, during which serum, plasma, and peripheral blood mononuclear cells are collected and stored. Additionally, extensive clinical and epidemiological information is collected on these study subjects. Nearly 200 subjects in the MACS have developed AIDS-lymphoma. Pre-cancer diagnosis plasma samples were obtained from MACS subjects who developed AIDS-lymphoma. Plasma also was obtained from subjects with AIDS who did not develop lymphoma. Using ELISA-type assays, we examined the expression of 17 chemokines and other markers in this plasma.

Results: Five markers showed a statistically significant elevation in the pre-lymphoma plasma, compared to the control plasma: three chemokines (CXCL13, ITAC and MIP-1 beta), a metallomatrix protein (MMP-9), and one hematopoietin-family cytokine (IL-10). Four other markers showed an elevation in those who went on to develop AIDS-lymphoma that was of borderline significance, including a chemokine (TARC) and three other molecules (ICAM-1, VCAM-1, and VEGF). Eight other markers showed no elevation pre-AIDS-lymphoma, including four chemokines (MIP-1 alpha, MIP-3 alpha, MIG, and IL-16), and four other molecules (IL-6, IL-12, sCD40L, and L-selectin). In other studies, we examined CXCR5 and CXCL13 expression in AIDS-lymphoma tumors, using immunohistochemistry. All AIDS-NHL tumors showed some degree of CXCR5 expression, and most (22/24) expressed CXCL13.

Conclusions: We have identified a number of chemokines and other markers that are potentially associated with AIDS-lymphoma. Studies to explore the role of chemokines in vivo, in a SCID mouse model, are underway.

Opportunistic Infections, Malignancies, and Model Systems

Interference of Probiotic lactobacilli with Candida albicans

Presenter: Gerwald Kohler, University of California, San Francisco

Principal Investigator: Gerwald Kohler

UARP Award Number: ID04-SF-030

Probiotics are live microorganisms that enhance health and well-being of man or animals by exerting beneficial effects on host mucosal surfaces of the gastrointestinal tract, the upper respiratory tract, or the urogenital tract. Some Lactobacillus species play a predominant role as advantageous components of the microflora, however, little is known about the molecular mechanisms these "good bacteria" use to interfere with pathogenic microorganisms. In order to elucidate how probiotic Lactobacillus species are able to directly suppress the growth of pathogens, we are using Candida albicans as a sensor organism. While commensal in healthy individuals, C. albicans has become the most important opportunistic fungal pathogen in the immunosuppressed causing severe mucosal or disseminated infections. DNA microarrays for C. albicans enable us to utilize genome-wide expression analysis to characterize the transcriptional response of the fungi to the presence of probiotic lactobacilli or their antimicrobial products. We have found that lactic acid production by the lactobacilli is a major, but not the only component inhibitory for fungal growth. Lactobacilli can generate oxidative stress responses in the fungi and appear in fact able to kill C. albicans cells. Consequently, they are also capable of interfering with formation of C. albicans biofilms. We also have gathered evidence that lactobacilli react to the presence of fungal cells and might actively induce components of their armamentarium to attack C. albicans. Our genomic approach will help to understand mechanisms of probiotic interference and results will provide a basis for further studies on the development of therapeutic regimens for opportunistic infections using probiotic bacteria. Additionally, the bacteria may also be employed for preventive measures, which could ultimately result in reduced transmission of HIV due to a healthy and robust mucosal flora.

Opportunistic Infections, Malignancies, and Model Systems

Elevated AID Expression in Peripheral Blood of HIV+ Pre-NHL Subjects

Presenter: Marta Epeldegui, University of California, Los Angeles

Collaborators: Yee Ping Hung, Elizabeth Crabb Breen, Roger Detels, Otoniel Martínez-Maza

Principal Investigator: Marta Epeldegui

UARP Award Number: D04-LA-403

Background: Non-Hodgkin's lymphoma (NHL) is the second most common cancer in AIDS patients, and is thought to result due to errors in class switch recombination (CSR) and somatic hypermutation (SHM) in B cells. Both processes are tightly regulated, occur in the germinal center (GC), and require the expression of the activation induced cytidine deaminase (AID) gene. Normally, AID is expressed only in GC B cells in secondary lymphoid organs, which is the site of CSR and SHM. The aberrant expression of AID (e.g., elevated and/or extranodal expression) could potentially lead to mistakes in the CSR and SHM processes, resulting in the mutation/translocation of proto-oncogenes, leading to the development of NHL. Since NHL is a common cancer in HIV infection and aberrant expression of AID could potentially lead to the development of lymphoma, we hypothesized that AID expression might be detectable in circulating cells in HIV infection, and preferentially elevated in those HIV+ subjects who went on to develop lymphoma.

Methods: In order to determine if AID was elevated in the peripheral circulation of HIV+ subjects who went on to develop lymphoma, AID expression was measured by quantitative real time PCR in peripheral blood mononuclear cells (PBMC) collected prior to lymphoma diagnosis; PBMC from HIV+ controls with AIDS but no lymphoma, and HIV-negative controls at comparable time points were also evaluated. This study was done using archival viably frozen PBMC from the Multicenter AIDS Cohort Study (MACS) at UCLA. The MACS is a study of the natural history of AIDS that was initiated more than 20 years ago. Subjects in the MACS are seen at two study visits per year, during which serum, plasma, and PBMC are collected and stored. Additionally, extensive clinical and epidemiological information is collected on these study subjects. PBMC, collected longitudinally over an extended period of time pre-NHL diagnosis, were tested from a total of 16 HIV+ lymphoma subjects at a study visit within 1 year prior to lymphoma diagnosis (n=14), between 1 to 5 years pre-lymphoma diagnosis (n=11), and more than 5 years prior to lymphoma diagnosis (n=11).

Results: It was seen that PBMC AID expression was undetectable in virtually all HIV-negative controls, but detectable at low to moderate levels in some of the HIV+/AIDS controls. Mean AID expression was significantly elevated at all three time points in those HIV+ subjects who developed lymphoma, with mean levels 100-1000 times higher compared to AIDS controls. When stratified by lymphoma subtype, it was seen that PBMC from those who developed central nervous system (CNS) lymphomas had little or no detectable AID expression, while moderate to high levels of AID expression were seen in the majority of those who developed non-CNS lymphomas (Burkitt's lymphoma and large cell lymphoma). Purified B cells from one lymphoma subject showed a 25-fold increase in AID expression compared to PBMC.

Conclusions: These results suggest that elevated levels of AID expression in circulating B cells precede lymphoma in HIV-infected persons, and support the hypothesis that aberrant AID expression may play a significant role in the development of AIDS-associated NHL.

Opportunistic Infections, Malignancies, and Model Systems

Chaperone-Templated Folding in Type III Secretion

Presenter: Loren Rodgers, University of California, San Diego

Collaborators: Roland Riek, Partho Ghosh

Principal Investigator: Loren Rodgers

UARP Award Number: D05-SD-401

Background: One of the hallmarks of advanced acquired immune deficiency syndrome (AIDS) is an increased susceptibility to bacterial pathogens, many of which utilize a type III secretion system (TTSS) to deliver toxin proteins (effectors) directly into the cytosol of host cells. Our long-term goal is to understand the steps required for protein transport by the TTSS. Here, we investigate the function of TTSS chaperone proteins. Inside the bacterium, TTSS chaperones bind one (or sometimes two) specific effector proteins. Although chaperones are not translocated into host cells, they are required for the translocation of their cognate effectors, and thus are central to virulence. Two alternative hypotheses have been proposed to describe the role of chaperones in type III secretion. The first hypothesis is that chaperones unfold their bound effectors, thereby maintaining them in a conformation that is competent for secretion. The alternative hypothesis suggests that chaperones mediate effector recognition by templating the formation of a three-dimensional motif that serves to target the effector to a component of the TTSS. Our specific aim is to determine how association of the Yersinia chaperone SycE affects the structure and dynamics of its corresponding effector YopE. If the chaperone were found to locally unfold the effector, this would provide direct evidence for the localized unfolding hypothesis. If instead the chaperone were found to locally fold the effector, this would support the hypothesis that structuring of the effector, as induced by the chaperone, may be required for interactions with bacterial components involved in TTSS transport.

Methods: Here, we use Nuclear Magnetic Resonance (NMR) Spectroscopy to compare an intact effector (YopE) in its free and chaperone-bound states, thereby determining how association with SycE affects the structure and dynamics of YopE.

Results: By using NMR spectroscopy we found that the chaperone-binding domain of YopE is flexible and lacks secondary structure in the "free" form, but becomes well-structured upon association with SycE.

Conclusions: These data demonstrate a "disorder to order" mode of chaperone action, thereby supporting translocation-targeting hypothesis.

Opportunistic Infections, Malignancies, and Model Systems

Candida albicans Modulates Host Defense by Synthesizing the Anti-inflammatory Lipid Resolvin E1

Presenter: Eric Haas-Stapleton, University of California, San Francisco

Collaborators: Song Hong, Yan Lu, Makoto Arita, Santosh Nigam, Charles N. Serhan, Nina Agabian

Principal Investigator: Eric Haas-Stapleton

UARP Award Number: F04-SF-211

Candida albicans is a commensal inhabitant of mucosal surfaces in immunocompetent individuals, but in patients with impaired immunity, this opportunistic fungus can invade tissues and cause life-threatening disease. We show that C. albicans cultured in the presence of the essential omega-3 fatty acid eicosapentaenoic acid (EPA) synthesizes several oxygenated products of EPA including resolvin E1 (RvE1), a lipid mediator of inflammation produced in humans by acetylated cyclooxygenase-2 (COX-2) or cytochrome 450 monoxygenase (CYP450) and 5-lipoxygenase (5-LO). Importantly, RvE1 promotes the resolution of inflammation in rodent models of dermal inflammation, peritonitis, and colitis. Although there is no known 5-LO equivalent in Candida, specific inhibitors of 5-LO markedly reduced fungal RvE1 synthesis without affecting growth. Human neutrophils, cells important for innate defense against pathogens, responded to nanomolar levels of RvE1 by exhibiting reduced IL8-stimulated chemotaxis, but enhanced phagocytosis and killing of Candida. Our results demonstrate that by producing a human immunomodulatory lipid, C. albicans can alter the host immune response, thereby potentially affecting fungal persistence in the host. Our finding that C. albicans can synthesize oxygenated lipids involved in the resolution of inflammation suggests that lipid-derived signaling molecules represent a new class of mediator that can function in the host-pathogen dialogue.

Opportunistic Infections, Malignancies, and Model Systems

Endogenous Nuclear Retrotransposition Antagonists of Ty3, a Retroviruslike Element in Budding Yeast

Presenter: Virginia Bilanchone, University of California, Irvine

Collaborators: Becky Irwin, Virginia W. Bilanchone

Principal Investigator: Suzanne Sandmeyer

UARP Award Number: ID05-I-008

Ty3 is a retroviruslike element in budding yeast which is studied as a model system for understanding the role of the host in supporting retroviruslike replication. A screen of 4500 yeast knockout mutants resulted in identification of 130 mutants affected (directly or indirectly) in Ty3 transposition. These were evenly divided between genes that positively and negatively affected transposition. Deletion of the checkpoint gene RAD24 negatively affected transposition, while deletion of a number of genes involved in DNA maintenance positively affected transposition. These include members of clamp loading complexes and proteins that interact with the replication clamp. Analysis of Ty3 protein and DNA intermediates in these mutants showed that in the case of a number of the DNA maintenance mutants the level of replicated Ty3 cDNA was increased. These data support a model where replicated cDNA may trigger a DNA damage response to Ty3 (as proposed for Ty1) which favors integration and perhaps repair of the integration site and that replication proteins may bind to and disrupt the Ty3 cDNA replication complex.

The laboratory is currently developing methods for ChIP analysis of Ty3 cDNA complexes. Using the yeast TAPtag collection in which individual epitope-tagged proteins are expressed from the native promoter, IgG beads will be used to precipitate various replication proteins and we will directly test for their association with Ty3 cDNA by Southern blot analysis of precipitates. We are also developing methods using the nuclease benzonase to better understand the change in particle structure that accompanies cDNA synthesis and nuclear entry. This information will be used together with knowledge of bound replication proteins to develop a model for the basis of the antagonistic effect of replication proteins on Ty3 cDNA.

Because many yeast proteins have mammalian homologs, the results of this study will suggest host proteins that may also interact with retrovirus preintegration complexes and be natural restriction factors to retroviral replication.

Innate Immunity and Host Factors

Antiviral Function of APOBEC3

Presenter: Nathaniel R. Landau, Salk Institute for Biological Studies

Collaborators: Darlene Chen, Renate König, Roberto Mariani, Derya Unutmaz, Qin Yu

Principal Investigator: Nathaniel R. Landau

UARP Award Number: ID04-SI-034

In the human genome the APOBEC3 gene has expanded into a tandem array of genes termed APOBEC3A-G. Two members of this family, APOBEC3G and APOBEC3F, have been found to have potent activity against vif-minus HIV-1. These enzymes become encapsidated in vif-minus HIV-1 virions and in the next round of infection, deaminate the newly synthesized reverse transcripts. The lentiviral Vif protein prevents the deamination by inducing the degradation of APOBEC3G and APOBEC3F. Our study showed that two additional APOBEC3 family members, APOBEC3B and APOBEC3C, have potent antiviral activity against SIV but not HIV-1. Both enzymes were encapsidated in HIV-1 and SIV virions and were active against vif-deleted SIVmac and SIVagm. SIV Vif neutralized the antiviral activity of APOBEC3C but not APOBEC3B. APOBEC3B induced abundant G-to-A mutations in both wild-type and vif-minus SIV reverse transcripts. APOBEC3C induced substantially fewer mutations. These findings raise the possibility that the different APOBEC3 family members function to neutralize specific lentiviruses.

Innate Immunity and Host Factors

Multiple APOBEC3 Family Cytidine Deaminases Are Potent Inhibitors of Mammalian Retrotransposons

Presenter: Nathaniel Landau, Salk Institute for Biological Studies

Collaborators: Qin Yu, Jody Chou, Nathaniel Landau

Principal Investigator: Hui Chen

UARP Award Number: F05-SIBS-212

Background: Retrotransposons are transposable endogenous retroviruses which are the driving force in evolution of the mammalian genome. Retrotransposons are classified as long terminal repeat (LTR) retrotransposons, such as intracisternal A-particle (IAP) and MusD and non-LTR retrotransposons, such as the LINE-1 elements. APOBEC3G was reported to inhibit the yeast LTR-retrotransposon Ty-1 and the mammalian MusD and IAP but not LINE-1. In this study, the ability of human and primate APOBEC3 family members to inhibit retrotransposition of MusD and IAP was tested.

Methods: A transfection based retrotransposition assay, mutational analysis and confocal microscopy were used to characterize effects of APOBEC proteins on retrotransposons. HeLa cells were cotransfected with APOBEC3 expression vector and neomycin-marked MusD and IAP and the resulting neomycin resistant clones were counted. G to A hypermutation was assessed by sequencing the retrotransposon using primers specific for the transposed DNA. The spliced form of the retrotransposons was amplified, cloned and sequenced. Cellular localization of the deaminases was determined by confocal microscopy. Cytidine deaminase activity using a 5'32P labeled oligonucleotide.

Results: APOBEC3A,B,C,F and G inhibited retrotransposition. The most potent inhibitor was APOBEC3A which was active in the nanogram range against both IAP and MusD. This was remarkable in that it was complete inactive against SIV when packaged at high copy number in virions. In contrast, APOBEC3G was nearly inactive on IAP. APOBEC3A was a potent cytidine deaminase in vitro and its activity against retrotransposons was dependent upon the zinc coordination residues and the catalytic glutamic acid. Although APOBEC3A was much more potent it appeared to cause fewer G to A mutations in the retrotransposons. APOBEC3A differed from APOBEC3G by being localized to the nucleus of the cell. This could play a role in its potency as an inhibitor.

Conclusions: The results further support a role for APOBEC cytidine deaminases in the inhibition of the endogenous retrotransposons. APOBEC3A is a potent inhibitor. This may be due to its cellular localization, small size and potent cytidine deaminase activity.

Innate Immunity and Host Factors

The Enhancement of Virus Release Activity of HIV-1 Vpu is Dependent on the AP-3 Pathway

Presenter: Beth Noble, Children's Hospital, Los Angeles

Collaborators: Juan Nuñez-Iglesias, Paula M. Cannon

Principal Investigator: Beth Noble

UARP Award Number: F05-CHLA-221

The assembly and release of retroviruses from human cells is inhibited by a dominant restriction factor. Different retroviruses have evolved mechanisms to counteract this factor, and this enhancement of virus release (EVR) activity is a property of the HIV-1 Vpu protein, the HIV-2 Env protein and the MLV Env protein. Although the EVR activity of Vpu was first described in 1989, the mechanism by which it enhances virus release is still unknown and represents a significant gap in our knowledge of HIV biology.

Previously, we have described a HeLa cell line (HeLa-T17) that has lost the ability to respond to Vpu, while remaining sensitive to stimulation by the HIV-2 Env. Confocal analysis revealed that the distribution of Vpu in these cells is aberrant, with a concentrated perinuclear distribution that colocalized with markers for the TGN and Golgi. To identify the defect in T17 cells we performed microarray analysis on wild-type and T17 HeLa cells, and observed that two different alkaline phosphatase (ALP) genes were highly up-regulated in the T17 cells. In yeast, ALP traffics from the Golgi to the large central vacuole through an interaction with AP-3, suggesting that the phenotype of T17 cells could arise from a defect in the equivalent mammalian pathway. To test this hypothesis, we performed RNAi knockdown of AP-3 in HeLa cells and found that this recapitulated the loss of Vpu activity. We also observed the same aberrant distribution of Vpu. Interestingly, in both T17 and AP-3 knock-down cells, the distribution of the CD63 marker was also altered. CD63 is present in late endosomes and lysosomes and uses AP-3 to traffic to these compartments from the TGN.

Previous studies have mapped the EVR function of Vpu to its membrane-spanning domain (MSD), a region known to have ion channel activity. Although no model has emerged to explain how ion channel activity could enhance HIV release this early observation has tended to focus studies on the MSD alone. We have now also found that the cytoplasmic tail of Vpu is essential for EVR activity, and mapped this to a [DE]XXXLq motif. Such sequences have previously been shown to interact with AP-1, AP-2, and AP-3 subunits. Mutation of residues within this motif both modified the protein's intracellular location and abrogated EVR activity, further suggesting that interaction with the correct cellular trafficking pathway is essential for Vpu's activity. Our current model for Vpu trafficking and activity will be discussed.

Innate Immunity and Host Factors

Counterregulation of APOBEC3G Enzymatic Activity in HIV Virions by Viral RNA and RNaseH

Presenter: Vanessa Soros, J. David Gladstone Institutes, San Francisco

Collaborators: Wes Yonemoto, Warner C. Greene

Principal Investigator: Vanessa Soros

UARP Award Number: F04-GI-220

Background: HIV-1 Vif is essential in vivo for it prevents incorporation of the antiviral enzyme APOBEC3G (A3G) into assembling virions. A deoxycytidine deaminase, A3G antiviral activity results in extensive dC ' dU deamination, effectively terminating viral replication. It has previously been demonstrated that cellular A3G is subject to regulation by assembly into a high molecular mass (HMM) complex. RNaseA treatment of HMM A3G generates low molecular mass (LMM) forms. Given that cellular HMM A3G is enzymatically inactive and that LMM A3G is enzymatically active, it was initially assumed that viral A3G, which we know is potently active during reverse transcription, would be incorporated into virions as the enzymatically active LMM form. These studies focus on the form and regulation of virion-packaged A3G.

Methods: Virions were produced by transient transfection of 293T cells, clarified and concentrated, and lysed under mild conditions. The virions were then subject to FPLC analysis followed by Western blot using antibody to A3G. Fractions identified as containing A3G were then subjected to A3G immunoprecipitation and the immunoprecipitates subjected to enzymatic analysis. Enzymatic tests assessed deoxycytidine deaminase activity and/or RNaseH activity.

Results: Surprisingly, FPLC analysis of virion lysate revealed that virion-incorporated A3G is in the HMM form. Furthermore, analysis of other virion components suggests that virion-incorporated A3G is in a complex containing IN and Vpr and viral genomic RNA. RNaseA-treatment of virion lysate results in redistribution of virion A3G into LMM complex forms. Since cellular HMM A3G is enzymatically inactive, we examined the activity of virion-derived HMM A3G. Similar to the activity profile for cellular A3G, immunoprecipitated virion HMM A3G is enzymatically inactive while LMM A3G derived from RNaseA-treated virions is enzymatically active. Furthermore, RNaseA treatment of the immunoprecipitated HMM A3G complex restored deaminase activity, indicating that the RNA component of the complex inhibits A3G activity. Similarly, A3G derived either from whole virion lysate or producer cell lysate is enzymatically inactive unless treated with RNaseA. Thus, we conclude from these observations that virion RNA inhibits A3G enzymatic activity.

Integral to the process of reverse transcription is removal of the RNA genome template by the RNaseH activity of Reverse Transcriptase (RT). A3G exerts its enzymatic antiviral activity during reverse transcription, suggesting that removal of RNA from virion A3G may be required for activation of its antiviral activity. When endogenous reverse transcription was initiated by incubation of lysed virions with dNTPs and Mg2+, A3G activity was indeed induced in the absence of any RNaseA addition. Importantly, addition of the RNaseH inhibitor, Compound I, during the incubation, completely inhibited A3G activation by the endogenous reverse transcription conditions. If the endogenous reverse transcription reactions were performed with virions that contain a point mutation in RNaseH rendering it inactive, A3G deaminase activity was no longer induced. These data strongly suggest that RNaseH activity is sufficient to activate HMM A3G derived from virions.

Conclusion: We propose that RNaseH is not only required for generation of the single-stranded DNA template that serves as a target for A3G deamination, but also "activates" virion A3G activity by degrading any associated inhibitory RNA from it, allowing it to now act on the ssDNA.

Innate Immunity and Host Factors

Cytokine-mediated Regulation of APOBEC3G Expression and Complex Formation in Primary Immune Cells

Presenter: Kimberly Stopak, J. David Gladstone Institutes, San Francisco

Collaborators: Jerry Kropp, Ya-Lin Chiu, Robert Grant, Warner C. Greene

Principal Investigator: Kimberly Stopak

UARP Award Number: D05-GI-413

Background: Human APOBEC3G (A3G), a deoxycytidine deaminase, is a broadly-acting antiretroviral factor. Given the antiviral effects of A3G, cytokine-stimulated T cells may upregulate A3G as a means to counter viral infection. Conversely, various cytokines such as IL-2, IL-4, IL-7, and IL-15 have been reported to convey permissivity to HIV infection in resting CD4 T cells. A3G can exist in two different forms, specifically an enzymatically-active low molecular mass form and an enzymatically inactive, high molecular mass A3G complex containing one or more RNAs. LMM A3G is characteristically found in resting CD4 T cells where it functions as a potent post-entry restriction factor, while HMM A3G complexes are found in mitogen-activated CD4 T cells (see Chiu et al., Nature 435:108, 2005). We have surveyed a range of cytokines to determine their effect on both A3G expression and A3G complex assembly.

Methods: Purified CD14- peripheral blood lymphocytes were stimulated individually with IL-2, IL-4, IL-6, IL-7, IL-9, IL-15, TNFalpha, and IFNalpha, beta, and gamma; and the potential induction of A3G mRNA and protein was assessed. Interferon-alpha-treated macrophages and mature versus immature dendritic cells were also assessed for potential changes in the expression of A3G. In parallel, the potential involvement of select signaling pathways in the observed response was tested by adding inhibitors specific for the Jak/Stat and MAP kinase pathways. Finally, using fast protein liquid chromatography, we assessed whether A3G was present in the LMM or HMM form after stimulation of purified CD4+ T cells with these cytokines.

Results: A3G expression at the protein level in PBLs was induced by IL-2 and IL-15, and, to a lesser extent, IL-7. No response was observed with IL-4, IL-6, IL-9, TNFalpha or the alpha, beta, or gamma isoforms of IFN. This increase in A3G protein was mirrored by upregulation of A3G mRNA detected by quantitative real time PCR. Inhibition of JAK or MAPK activation sharply impaired the induction of A3G in response to IL-2, IL-7, and IL-15. Dendritic cell maturation and interferon-treatment of macrophages was also associated with a marked increase in the expression of A3G. When A3G complex formation was assessed, IL-2, IL-7 and IL-15 each promoted HMM A3G complex assembly in purified CD4 T cells.

Conclusion: We conclude that IL-2, IL-7, or IL-15 stimulation of resting CD4 T cells leads to transcriptional activation of the A3G gene resulting in increased A3G mRNA and protein expression. However, the potent post entry restricting activity of the LMM form of A3G is forfeited because the induced A3G protein is recruited into inactive HMM A3G RNA-protein complexes. These effects may explain why these cytokines cause resting CD4 T cells to become permissive to HIV-1 infection.

Innate Immunity and Host Factors

Determinants of HIV-1 Restriction by the A3G Complex

Presenter: Mario Santiago, J. David Gladstone Institutes, San Francisco

Collaborator: Warner C. Greene

Principal Investigator: Mario Santiago

UARP Award Number: F05-GI-225

Background: The dynamic interplay between viral proteins and innate host factors represent promising avenues for novel antiviral intervention strategies. Recently, our laboratory found that A3G is a potent restriction factor for HIV-1 infection of resting T-cells, and this antiviral activity correlated with its recruitment into low- (LMM) and high-molecular mass (HMM) complexes (Chiu et al. 2005 Nature 435(7038):108-14). The LMM complex functions as a potent antiretroviral barrier in unstimulated T cells, but upon T cell activation, A3G is recruited into an enzymatically-inactive HMM ribonucleoprotein complex that is incapable of restricting single-round HIV-1 infection. Intriguingly, the ability of the A3G LMM complex to block HIV-1 infection in resting T-cells does not seem to solely depend on its deaminase activity.

Methods: To investigate the structural and functional determinants of A3G that govern HMM complex formation and HIV-1 restriction, we propose to generate a panel of A3G mutants that takes into account the potential functional redundancy imposed by A3G's double-domain structure. These 'double-mutants' will then be characterized for their ability to form HMM complexes and mediate the HIV-1 post-entry block in permissive cells. These properties will then be correlated with RNA binding, deaminase activity, dimer formation and ability to form cytoplasmic bodies.

Expected Results: We hope to identify A3G mutations that will disrupt HMM complex formation. Such mutants that constitutively express in the LMM form may effectively restrict HIV-1 infection in permissive cells. If so, the properties of these mutants that facilitate the antiviral activity will be addressed by further mutagenesis.

Conclusions: Unraveling the structural domains of A3G that govern HMM complex formation and HIV-1 restriction may provide a much-needed molecular blueprint for exploiting this innate restriction factor as a therapeutic agent against HIV-1.

Innate Immunity and Host Factors

A Serine/Threonine Kinase Activity Associates with the N-terminus of Simian Immunodeficiency Virus Nef

Presenter: Erwin Antonio, University of California, Davis

Collaborators: Scott Wong, Kaitlin Thompkins, Balvinder Rehal, Cintia Ho, Gary Rhodes

Principal Investigator: Earl Sawai

UARP Award Number: ID03-D-060

Background: The myristylated Nef protein of HIV and simian immunodeficiency virus (SIV) has been shown to be an important determinant of high levels of viremia and unperturbed progression of AIDS in HIV-infected human patients and SIV-infected experimental rhesus monkeys. The putative role of Nef as a multifunctional, adaptor protein involves a variety of biological activities demonstrated in vitro, including CD4 and MHC I downregulation, modulation of cell signaling activity (i.e. p21-activated kinase (PAK) activation), and enhancement of virion infectivity. In this study, we analyze CD8-HIV/SIV Nef chimeras in in vitro kinase assays to examine differences in the genetic determinants of HIV Nef- and SIV Nef-induced kinase activity.

Methods: DNA expression constructs encoding CD8-fusion proteins of HIV/SIV Nef chimeras or deletion and point mutations in SIV Nef were cloned using PCR mutagenesis. The resulting plasmids were transiently-transfected into COS-7 cells to express Nef and in vitro kinase assays performed on Nef immunoprecipitates. Kinase assay profiles were resolved by SDS-PAGE and visualized by autoradiography.

Results: We identify a previously unrecognized serine/threonine kinase activity that associates with SIV Nef but not HIV-1 Nef. This kinase activity is enhanced for CD8-Nef fusion proteins but also detected for native Nef. We find this kinase activity maps to the N-terminus of SIV Nef and results in the phosphorylation of a unique ~49 kDa substrate that is distinct from Nef and PAK. Moreover, amino acid substitution of specific tyrosine residues within the N-terminus of SIV Nef impairs phosphorylation of p49. Interestingly, we have identified strong sequence homology between the N-terminii of SIV Nef and Matrix (MA) of the Mason-Pfizer Monkey Virus (MPMV), and find that genetic determinants of the putative myristyl switch function of MPMV MA are homologous to those of SIV Nef important for p49 phosphorylation.

Conclusion: We have identified a novel function associated with SIV Nef. This function represents a previously unrecognized cellular kinase activity that we designate the SIV Nef N-terminal kinase (SNTK) activity. The SNTK activity results in serine and threonine phosphorylation of p49, yet the identity of this substrate remains to be determined. Moreover, homology between the SNTK-interaction domain of SIV Nef and the N-terminus of MPMV MA suggests that the SNTK activity may be linked to a myristyl switch mechanism.

Opportunistic Infections & Related Topics

Novel Drug Targets for Toxoplasmosis

Presenter: Sharon Reed, University of California, San Diego

Principal Investigator: Sharon Reed

UARP Award Number: ID04-SD-079

Toxoplasma gondii encephalitis is a common cause of central nervous system infection in patients with AIDS and is uniformly fatal unless diagnosed and treated early. Many patients cannot tolerate the current optimal treatment regimens of high dose sulfa drugs because of serious allergic reactions. Thus, further understanding of the pathogenesis of infection by T. gondii and the identification of potential drug targets is critical. We have cloned the only cathepsin B gene (TgCPB), purified native and recombinant enzymes, and shown that this proteinase localizes to the rhopteries, an organelle essential to invade humans and cause disease. Chemical nhibitors of TgCPB and antisense RNA to disrupt TgCPB expression limit toxoplasma invasion, intracellular multiplication, and disseminated infection in our new chick embryo model.

We have chosen the vinyl sulfone inhibitor, K11777, to use as a scaffold for drug design because it is effective in both in vitro and in vivo models of toxoplasma invasion and is cell permeable, which is critical for an intracellular parasite. Furthermore, it has undergone extensive metabolism and bioavailabilty testing and therefore much more is known about the physicochemical properties of this inhibitor than any other inhibitor of T. gondii cathepsin B. To begin to develop inhibitors of the parasite cathepsin B, we have conducted initial drug screening against the recombinant parasite cathepsins B and L as compared to their effects on human cathepsin B. For these studies, rTgCPB and rTgCPL of T. gondii were expressed in Pichia and the activity was determined by detection of the fluorescent cleavage product of the substrate, Z-Arg-Arg--AMC (10 uM).

K11777 is the most potent of all of the compounds we have tested against T. gondii cathepsin B with an IC50 value of approximately 140 nM and is 2-fold more potent at inhibiting the parasite cathepsin B versus the human homolog. Importantly, an analog of K11777, K11002, which has only a slight structural difference from K11777 is over 100-fold less potent at inhibiting T. gondii cathepsin B and shows higher affinity for human versus the parasite cathepsin B. The finding that small variations in the K11777 structure result in major differences in potency and specificity at inhibiting the T. gondii cathepsin B suggests that we should be able to use medicinal chemistry to identify compounds with even greater potency and selectivity at inhibiting T. gondii cathepsin B than K11777. Our studies support the importance of cathepsin B as a valid drug target as well as the feasibility of translating our findings into viable drugs.

Opportunistic Infections & Related Topics

US15, a Cell Specific Suppressor of HCMV Infection in Retinal Pigment Epithelial Cells (RPE)

Presenter: Rong Hai, University of California, Berkeley

Principal Investigator: Rong Hai

UARP Award Number: D04-B-405

Background: Human cytomegalovirus (HCMV) retinitis is an important etiology of blindness in AIDS patients. The mechanism of HCMV pathogenesis in the retina is unknown; however, the retinal pigment epithelium (RPE) cells play a key role in AIDS retinitis and are the target of HCMV infection in vivo. The ability of HCMV to infect and replicate in RPE cells is responsible for the development of viral-associated retinitis. Similar to that in vivo, RPE cells are fully permissive to HCMV infection in vitro.

Method: To understand HCMV replication in RPE cells, the HCMV genome was cloned as a bacterial artificial chromosome (BAC) to generate a deletion mutant library. We systematically investigated the necessity of each ORF for replication in RPE cell through phenotypic screening of growth properties in RPE cells. A revertant virus has been constructed to confirm whether the enhanced phenotype is specifically due to deletion of US15. For further unveiling the molecular mechanism of this suppression, Western analysis and microarray study were being carried through.

Results: Compared to wild type BAC, US15 mutant displayed an enhanced growth phenotype. In contrast, the growth of US15 mutant in human foreskin fibroblasts was not significantly different from that of the parental HCMV-BAC. The revertant virus confirmed that the enhanced phenotype is specifically due to deletion of US15. Western analysis showed that since alpha phase of viral life cycle the viral protein expression level of US15 mutant was higher than the parental HCMV-BAC. Further microarray study showed that the US15 mutant had higher viral transcription level from the immediate early stage of viral life cycle comparing to the parental HCMV-BAC.

Conclusion: Through the above study it is clear that the suppression effect of US15 happens during viral life cycle alpha phase and is due to the suppression of viral immediate early genes transcript. This useful information will help us to develop innovative anti-viral strategies for HCMV retinitis.

Opportunistic Infections & Related Topics

Identification of a Secreted Protein Kinase Used by Toxoplasma to Alter Host Gene Expression

Presenter: Susan Coller, Stanford University

Collaborators: Jeroen P. Saeij, John C. Boothroyd

Principal Investigator: Susan Coller

UARP Award Number: F04-ST-207

Background: Toxoplasma gondii is an opportunistic pathogen that can cause life-threatening encephalitis in patients with compromised immune systems, such as those suffering from HIV/AIDS or undergoing chemotherapy for treatment of cancer. Toxoplasma is an obligate intracellular parasite that establishes infection in virtually any nucleated cell within the mammalian host. To date, the mechanisms by which Toxoplasma is able to manipulate the host cell machinery and create a suitable environment in which to replicate are largely unknown. Recently, it has been demonstrated that at least one protein originating from the specialized secretory organelles, termed rhoptries, can be localized within the host cell nucleus. These results suggest that Toxoplasma rhoptry proteins have the potential to directly interact with host cell molecules and may be responsible for allowing the parasite to grow within the host cell. Our hypothesis is that some of these parasite proteins will have direct regulatory effects on the host cell substrate targets. One category of protein enzymes, termed kinases, have been historically shown to be critical for the regulation of numerous cellular processes in virtually all eukaryotic cell types. The overall goal of my work is to identify Toxoplasma kinases that are responsible for overcoming the host cell's natural defenses and which allow for successful parasite infection. Currently, the focus of my research is to identify parasite kinases that have the ability to directly interact with host cell molecules and alter their normal patterns of functioning.

Methods: The proteins that are trafficked to the rhoptries have in common a specific and recognizable amino acid sequence that targets them to this organelle. We used the desire to identify parasite kinases important for host cell invasion combined with the required trafficking signature as two criteria to create a list of candidate proteins from those predicted by the Toxoplasma genome database. In all, we identified approximately twenty putative Toxoplasma proteins that met the above criteria. One of these proteins was independently identified in a QTL analysis and mapped to a specific region in the parasite genome that correlated to significant regulation of host cell gene transcription. Recently, it was also identified in a proteomic analysis of the rhoptries and designated ROP16.

Results & Conclusions: We have begun characterizing ROP16, and have demonstrated that it is secreted into the host cell during the invasion process and interestingly, localizes to the host cell nucleus. We are currently testing whether the putative kinase domain is enzymatically functional and identifying any host cell substrates that may be interacting with ROP16. This is the first demonstration of a Toxoplasma kinase that is not only secreted into the host cell, but also is trafficked into the host cell nucleus. To date, attempts to genetically disrupt ROP16 in Toxoplasma have been unsuccessful, suggesting that it may be an essential component in the parasite's ability to infect host cells. Thus ROP16 has the potential to be an important element in subverting the host cell's natural defenses and allowing for unchecked parasite replication and may be a novel target of drug therapy.

Opportunistic Infections & Related Topics

Role of Vomp Adhesins in Bartonella quintana Pathogenesis

Presenter: Joanna MacKichan, University of California, San Francisco

Collaborator: Jane E. Koehler

Principal Investigator: Joanna MacKichan

UARP Award Number: F05-SF-205

Background: Bartonella quintana (BQ) is a louse-borne pathogen that can persist for months in the human bloodstream. BQ causes opportunistic infections in HIV-infected patients, producing relapsing fever (trench fever), endocarditis, bacillary angiomatosis, and chronic bacteremia. Relapsing and/or chronic bloodstream infection occurs in patients at all stages of HIV infection and can last for months to more than a year, causing debilitating and even fatal sequelae. When BQ gains access to the human bloodstream, some bacteria remain free and some invade and reside within red blood cells. An additional persistence strategy is suggested by the relapsing pattern of fever observed during BQ infection: phase variation of antigenic surface proteins, enabling escape from the host immune system. Recently, our lab demonstrated that a novel family of BQ outer membrane protein (OMP) adhesins, designated Vomp (Variably-expressed OMP), undergoes phase variation. The four Vomp, homologous to the adhesin YadA from Yersinia, were expressed in the strain (a clinical isolate, JK-31) used to inoculate our animal model, but were no longer expressed in isolates from the bloodstream 70 days post-inoculation. Preliminary data suggest that the Vomp are major virulence factors, with collagen binding and autoaggregation functions.

Methods: Using a negative selection method, we constructed a vomp null mutant in BQ. A mutagenic plasmid, containing sequences flanking the vomp locus, was conjugated into JK-31, and colonies containing the integrated plasmid were selected. A subsequent selection, for strains that had lost the integrated plasmid, was performed. Approximately one third of the strains had lost the vomp locus along with the plasmid. The vomp null mutant was verified by colony PCR, Western, and Southern blot. The mutant is currently being complemented, both with each of the genes individually (with their native promoters), and with the entire vomp locus. In each case, the complementing gene or region is being amplified by PCR and cloned into the replicating plasmid pANT3. An autoaggregation assay, to determine the rate at which BQ falls out of suspension, was done for the mutant as well. The vomp null mutant was then tested for virulence in an animal model in which BQ wild type inoculation results in bloodstream infection 100% of the time.

Results: The vomp null mutant was confirmed by PCR, Western blot, and Southern blotting techniques. Complementation of the vomp null mutant with each of the individual vomp genes, or with the entire vomp locus, is currently ongoing. The vomp null mutant has been found to be defective for autoaggregation, suggesting that the Vomp function as autoadhesins. In addition, while every strain of wild type BQ that has been used to infect an animal model can be recovered from the bloodstream, the mutant could not be recovered. This confirms that the Vomp are important virulence factors, and are essential for infection.

Conclusion: This is the first time that targeted mutagenesis has been achieved in BQ. The lack of genetic tools has hindered the study of this emerging opportunistic pathogen. The vomp mutant has been found to be deficient for autoagglutination, a virulence phenotype, as well as for infection in an animal model. This suggests that the Vomp are important virulence factors for BQ. The vomp null mutant and complemented strains will be used to further address the role of the individual Vomp in BQ pathogenesis.

Opportunistic Infections & Related Topics

Using an mRNA Assay to Detect Trimethoprim-Sulfamethoxazole Drug Resistance

Presenter: Laurence Huang, University of California, San Francisco

Principal Investigator: Laurence Huang

UARP Award Number: ID04-SF-026

Background: Pneumocystis pneumonia (PCP) remains a significant cause of morbidity and mortality in HIV-infected persons. Trimethoprim-sulfamethoxazole (TMP-SMX) is the first-line PCP treatment and also the first-line PCP prophylaxis regimen. The widespread use of TMP-SMX for PCP prophylaxis has raised increasing concerns regarding the development of drug-resistant organisms. Since P. jirovecii cannot be cultured, standard microbiologic methods to detect drug resistance cannot be used. SMX, responsible for most of the anti-pneumocystis activity of TMP-SMX, inhibits the P. jirovecii (formerly P. carinii) dihydropteroate synthase (DHPS) enzyme. Several lines of evidence have led researchers to study DHPS mutations as a possible mechanism for TMP-SMX resistance. Recently, a reverse transcriptase polymerase chain reaction (RT-PCR) assay based on detection of the Phsb1 transcript of human Pneumocystis has been developed. The assay was shown to distinguish between viable and non-viable, heat-killed Pneumocystis and was suggested as a surrogate viability assay. This study explores the use of this RT-PCR mRNA assay as a serial measure of response to therapy, and aims to correlate this response with DHPS genotype and PCP treatment as an indicator of putative TMP-SMX drug resistance.

Objectives: (1) To correlate serial mRNA results with clinical outcomes, including PCP treatment response and mortality, in patients with PCP; (2) To correlate P. jirovecii molecular genotype at the DHPS locus with serial mRNA results in patients with PCP.

Methods: We performed a cross-sectional hospital-based study. Subjects were HIV-infected patients with PCP diagnosed by sputum induction and/or bronchoscopy with bronchoalveolar lavage (BAL). These sputum and BAL specimens were PCR-amplified and DNA sequenced at the DHPS locus. Subjects provided serial oropharyngeal wash (OPW, gargle) specimens by gargling 10 ml 0.9% NaCl for 60 seconds daily during their hospitalization. The OPW specimen was examined using the RT-PR mRNA assay. Standardized chart abstraction was performed to record the specific PCP treatment and to determine important clinical outcomes at 6 weeks, including death, death attributable to PCP, and PCP treatment failure.

Results: To date, we have enrolled 25 HIV-infected patients with PCP. Eighteen (72%) of the 25 subjects had Pneumocystis DHPS mutations, despite the fact that only six (24%) subjects were receiving TMP-SMX (or dapsone, a sulfone that, like SMX, is a DHPS inhibitor) for PCP prophylaxis within the preceding 3 months. All of the patients were treated with TMP-SMX. All responded to therapy, were discharged from the hospital, and remain well at 6 weeks. At present, RT-PCR mRNA analysis of the OPW specimens is in progress.

Conclusion: Although the majority of HIV-infected patients diagnosed with PCP at San Francisco General Hospital have Pneumocystis containing DHPS mutations, the proposed mechanism underlying putative TMP-SMX drug resistance, these subjects appear to respond to TMP-SMX treatment for their PCP.

Opportunistic Infections & Related Topics

Mapping of Parasite Genes Responsible for Differences in Host Responses to Different Toxoplasma Strains and Identification of the Host Transcription Factors Involved

Presenter: Jeroen Saeij, Stanford University

Collaborators: J. P. Boyle, Susan Coller, John C. Boothroyd

Principal Investigator: Jeroen Saeij

UARP Award Number: F04-ST-216

During the initial stages of an infection with Toxoplasma gondii, 'tachyzoites' (the rapidly dividing form of the parasite) disseminate throughout the host tissues, including the brain. A robust immune response during this acute phase eliminates most tachyzoites; however, some parasites convert to encysted 'bradyzoites' that may persist for the life of the host and are mainly located in the brain. Toxoplasma infection is a constant threat for immunocompromised individuals, such as AIDS patients, because reactivation leads to eruption of the cysts in the brain leading to life-threatening Toxoplasmic encephalitis. An improved understanding of how Toxoplasma establishes a persistent infection in healthy individuals and reactivates in AIDS will help to contribute to the development of effective therapeutic strategies.

My research focuses on determining how Toxoplasma manipulates its host enabling it to establish and maintain a life-long persistent infection and then later reactivate and cause problems in AIDS patients. I am using Toxoplasma strains that differ in their ability to establish a persistent infection to investigate this question. Microarray analysis of host gene expression of human cells infected with different Toxoplasma strains shows dramatic differences in the ability of different strains to modulate subsets of host genes. Using similar analyses of cells infected with progeny derived from a cross between type II and type III strains, we have genetically mapped the parasite loci responsible for these differences. Surprisingly, the data indicate a single Mendelian locus is responsible for each of the differences in phenotype. Candidate genes from these regions are currently being investigated and will be discussed. In addition, bioinformatic analysis of the host genes that are coordinately regulated by infection with the different parasite strains indicates that many appear to be regulated by the same transcription factors. Immunofluorescence assays and Western blots have been used to confirm the role of these molecules. Hence, we now have a handle on both the host and parasite molecules that mediate strain-specific differences in host response to infection. The next step will be to investigate the role of these molecules in helping Toxoplasma to establish a persistent infection.

Opportunistic Infections & Related Topics

Essential Functions of Virion Tegument Proteins Revealed in a Model Gamma-Herpesvirus

Presenter: Eric Bortz

Collaborators: Shaoying Lee, Seunming Hwang, Nichole Reyes, Ting-Ting Wu, Hongyu Deng, Qingmei Jia, Hong Zhou, Julian Whitelegge

Principal Investigator: Ren Sun

UARP Award Number: ID04-LA-085

Background: The gamma-herpes viruses, Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), are associated with a number of human malignancies, particularly in patients with AIDS. Transmission of these viruses to B-lymphocytes, the primary cell type in which EBV and KSHV establish latency, is thought to be dependent upon production and release of infectious virus particles, or virions, culminating the lytic phase. Murine herpesvirus-68 (MHV-68) is a robust in vitro and in vivo model for gamma-herpesvirus infection. Unlike EBV and KSHV, MHV-68 produces high titers of infectious virions in tissue culture and is capable of establishing both acute and latent infections in laboratory mice. We are studying the structure and protein composition of the MHV-68 virion and have identified two gamma-herpesvirus specific proteins, ORF45 and ORF52, that are critical mediators of virion infectivity. These proteins are found in the virion tegument, a poorly understood structure encasing the nucleocapsid and underlying the virion envelope.

Methods: Virions and infected cell ultrastructures were studied by cryo-electron microscopy, cryo-electron tomography, and transmission electron microscopy (TEM). Virion proteins were analyzed by immunoblotting, mass spectrometry (LC/MS-MS), and molecular cloning into FLAG-tagged, enhanced green fluorescent protein (EGFP), or dsRed fluorescent protein expression plasmids. Site-specific nonsense mutations in genes encoding tegument proteins were introduced into an MHV-68 genome cloned as a bacterial artificial chromosome (BAC) by homologous recombination with a shuttle plasmid in E. coli. Mutants were analyzed by virological assays and complementation in mammalian cells.

Results: The ORF52 protein is more tightly bound to the nucleocapsid than the ORF45 protein, suggesting that ORF52 localizes to a semi-ordered inner layer, and ORF45 to a more labile outer tegument layer. Cryo-electron tomographic reconstructions of MHV-68 virions support the existence of two distinct tegument layers. While the ORF45 protein displays predominantly nuclear localization, ORF52 protein localizes to a cytoplasmic compartment partly overlapping with the trans-Golgi network and actin cytoskeleton. Mutagenesis of ORF45 and ORF52 in an MHV-68/BAC clone revealed that both proteins are required to the complete the lytic phase of infection. Viral lytic gene expression and DNA replication is severely attenuated by loss of ORF45. In the absence of ORF52 protein, the lytic phase arrests after replication of viral DNA, expression of late lytic genes, and assembly and packaging of capsids but prior to tegumentation and envelopment of nascent nucleopcapsids in the cytoplasm.

Conclusions: Thus, while ORF45 protein is required in the immediate-early stage of infection, ORF52 protein functions to facilitate virion morphogenesis and egress. The protein-protein interactions through which ORF45 and ORF52 function and are packaged into the virion tegument are under investigation, as well as indications that the proteins interact with each other. Elucidating the structure-function relationships making up the gamma-herpesvirus virion tegument develops a potential target for therapeutic interventions aimed at preventing the release of infectious virus from the cell.

Latency and Viral Mechanisms

Functional Roles of Human Immunodeficiency Virus Type 1 Integrase During Reverse Transcription

Presenter: Samson Chow, University of California, Los Angeles

Collaborators: Charles Dobard, Thomas Wilkinson

Principal Investigator: Samson Chow

UARP Award Number: ID05-LA-021

After cell entry, the RNA genome of retroviruses is converted to a cDNA copy by reverse transcriptase (RT). This viral cDNA, incorporated into a nucleoprotein complex termed the preintegration complex, enters the nucleus and is integrated into the host cell chromosome. Retroviral integrase (IN) catalyzes the integration of viral DNA. Both reverse transcription and integration are essential steps in the viral life cycle. The function of IN is not restricted to integration. For human immunodeficiency virus type 1 (HIV-1) certain mutations of IN, such as substituting the Cys residue at position 130 of IN to Ser (NL-C130S), can cause a specific defect in reverse transcription, but the underlying mechanism for this defect is poorly understood. In vitro, IN and RT can physically interact, and we have determined previously by co-immunoprecipitation that HIV-1 RT interacts with the C-terminal domain of IN. We hypothesize that the IN-RT interaction is functional and critical for reverse transcription. To confirm that the lack of the reverse transcription is caused by the IN mutation, wild type (WT) IN was packaged into the NL-C130S mutant virions by the in-trans method. Incorporation of IN restored reverse transcription to 25% of the WT virus. To understand the mechanism by which IN stimulates reverse transcription, we employed cell-free reverse transcription assays to examine the effects of IN on the RNA- and DNA-dependent polymerase activity of RT. Our preliminary results showed that IN can stimulate both the initiation and elongation mode of reverse transcription. In the presence of 10 molar excess IN, initiation and elongation products increased nearly 3-and 2-fold, respectively. In addition to initiation and elongation, IN may help stimulate the early steps of reverse transcription by promoting annealing of the tRNA primer to the template, stabilizing the RT-template-primer ternary complex, or destabilizing secondary structures of the RNA primer and template, all of which could lead to an increase in initiation and extension products. We are currently investigating such possibilities with aims to identify the critical step that IN is required during early events of reverse transcription. The experiments will shed light on the interaction between two key retroviral enzymes and the effect of such an interaction on the essential step of reverse transcription. Characterizing the RT-IN interaction and determining its biological significance may reveal new functional roles for IN as well as potential targets for devising new approaches to inhibit viral replication.

Latency and Viral Mechanisms

Inducible Expression of Small Interfering RNAs to Inhibit HIV Replication

Presenter: Jing-Kuan Yee, Beckman Research Institute of the City of Hope

Collaborators: Hsin-Lung Lo, Tammy Chang

Principal Investigator: Jing-Kuan Yee

UARP Award Number: ID05-BRI-067 Yee

Background: Gene therapy strategies using genetically modified T cells or hematopoietic stem cells (HSCs) have been proposed as an adjuvant to chemotherapy for treating HIV infection. The discovery that exogenously delivered siRNA can trigger RNAi in mammalian cells raises the possibility of harnessing RNA interference (RNAi) technology as a therapeutic tool against HIV infection. This is confirmed by stable expression of short hairpin RNAs (shRNAs) via HIV vector-mediated gene transfer in T cells, rendering cells resistant to HIV replication. However, HIV resistant strains with mutations in the siRNA target site emerge quickly. Thus, expression of siRNAs against multiple targets in the HIV genome may be necessary to effectively suppress the emergence of resistant strains. Using multiple pol II promoters in a HIV vector to drive siRNA expression is technically difficult. Using multiple pol III promoters is feasible but constitutive siRNA expression from strong pol III promoters will most likely generate undesirable "off-target" effect in the target cell. In contrast to siRNA, multiple microRNAs (miRNAs) can be expressed as a single transcript from a pol II promoter and can be processed efficiently by Drosha based on their unique stem-loop structures.

Methods: We propose to evaluate whether multiple siRNAs with each siRNA flanked by the stem-loop structure of pri-miR-30a can be expressed and processed properly from a pol II promoter-derived polycistronic transcript. We will determine whether inducible pol II promoters can be used to drive the expression of multiple siRNAs against HIV in the target cells.

Results or expected results: We have so far inserted an shRNA gene against a common exon for the tat and rev genes into the stem-loop structure of pri-miR-30a. A constitutive pol II promoter and the GFP gene were placed upstream from the shRNA gene and the whole cassette was then inserted into a HIV vector. H9 cells were transduced with this vector and challenged with HIV-1. We will determine whether the siRNA is properly processed and whether it can render the cell resistance to HIV-1 replication. If this design allows efficient expression and proper processing of this siRNA, we will evaluate whether inserting two shRNA genes targeted to different regions of the HIV genome can lead to a similar efficiency of expression and processing. We will then determine whether the well-characterized human interleukin 2 (IL-2) promoter can drive T cell-specific and inducible expression of the siRNA genes. We will also evaluate whether HIV long terminal repeat (LTR) which responses strongly to the HIV Tat protein can also serve as an inducible promoter for siRNA expression.

Latency and Viral Mechanisms

Assembly of NFkappaB on the HIV-LTR

Presenter: Amanda Fusco, University of California, San Diego

Principal Investigator: Amanda Fusco

UARP Award Number: D04-SD-404

The maintenance of human immunodeficiency virus (HIV) life cycle in infected host human cells requires host proteins. A class of transcription factors known as nuclear factor kappaB (NF-kappaB) is among the host proteins that HIV uses for its gene expression. The NF-kappaB family of dimeric transcription factors regulates the expression of genes that are key to a variety of biological responses. NF-kappaB dimers are formed by the combinatorial association of five members, p50, p52, RelA, c-Rel and RelB. It is known that these dimers bind to a class of DNA sequences (kappaB sites) located within the promoter/enhancer region of a large number of response genes. The HIV-LTR contains such kappaB sites, which have been shown to utilize the NF-kappaB/Rel proteins to regulate transcription through binding to these kB sites. One of the HIV isolates contains two such kappaB sequences arranged in tandem. Binding of two NF-kappaB dimers to these tandem sites is essential for synergistic transcriptional activation from HIV-LTR. Surprisingly, our biochemical experiments have shown that NF-kappaB dimers fail to bind to specific sequences arranged in tandem with high affinity. I will investigate if the transcriptional coactivator (CBP) binds directly and simultaneously to both NF-kappaB molecules and thereby promoting stable association of CBP and NF-kappaB on the HIV-LTR. This is thought to play a role in relieving the anti-cooperative effects of NF-kappaB bound to tandem kappaB sites. Different biochemical techniques such as electrophoretic mobility shift assay (EMSA), fluorescence anisotropy assay (FAA) and affinity pull down assays will be utilized to demonstrate the assembly of CBP and NF-kappaB on different DNA fragments taken from various HIV strains.

Recent studies have shown that two different NF-kappaB dimers are responsible for the regulation of HIV gene expression in different cells; NF-kappaB p50/p65 primarily acts in CD4+ T cells and NF-kappaB p52/RelB heterodimer acts in monocyte and macrophage cells. Whereas the molecular structure of HIV-DNA bound p50/p65 hetrodimer is known, no such structure is known for the other dimer bound to HIV-DNA. Large crystals of p52/RelB complexed to DNA have been obtained. The crystals have diffracted to 3Å and the X-ray diffraction data has been collected and processed. During the process of improving the crystals I determined that p52/RelB binds to DNA in a different manner than other NF-kappaB family members. Therefore determining the structure will be essential before I can proceed with the cooperative effects of tandem kappaB sites.

Latency and Viral Mechanisms

Murr1 Inhibition of NF-kappaB-dependent HIV Transcription

Presenter: Tom Huxford, San Diego State University Research Foundation

Principal Investigator: Tom Huxford

UARP Award Number: ID05-SDSUF-014

Background: In addition to the genes encoded by HIV on the viral genome, HIV depends upon host cellular factors and processes throughout its life cycle. The development of drug therapies that interfere with these cellular processes is vital as they represent immutable targets. HIV relies heavily upon host cell machinery in activating expression of latent viral DNA that is integrated within transcriptionally silent regions of heterochromatin. The virus accomplishes this by using the strong transcriptional potential of NF-kappaB, a master transcription factor that becomes activated in response to signals produced by normal viral infection of immune cells. The transition of NF-kappaB from its inactive to active state requires the phosphorylation and ubiquitin-dependent proteolysis of its inhibitor, IkappaB. The small protein Murr1 has been shown to interfere with this process by interacting with inactive NF-kappaB/IkappaBalpha complexes and impeding ubiquitinylation of the inhibitor. As a result of this activity, Murr1 disrupts the ability of NF-kappaB to activate transcription from the long terminal repeat (LTR) HIV promoter. Structural and biochemical characterization of these factors could lead to the discovery of a new strategy, complementary to the existing therapies aimed at inhibition of viral enzymes, to maintain HIV in its latent state by targeting host cell factors.

Methods: We have successfully expressed and purified to homgeneity milligram amounts of recombinant Murr1 protein as well as the NF-kappaB p50 and p65 subunits and IkappaBalpha. The folded structure of Murr1 will be probed by controlled proteolysis and mass spectrometry. As Murr1 was original cloned and characterized as a gene involved in copper metabolism, the metal-binding properties of Murr1 will be assessed. Finally, the ability of Murr1 to undergo phosphorylation by protein kinase CK2 (casein kinase II) will be quantitated and its effects on Murr1 structure and function will be assessed. Next, three independent methods for determining Murr1 binding affinity to inactive NF-kappaB/IkappaBalpha complexes will be employed. A new in vitro functional assay will be developed which directly measures the ability of Murr1 to inhibit ubiquitinylation of NF-kappaB/IkappaBalpha. Finally, the three-dimensional folded structure of Murr1 both in its free state and in complex with NF-kappaB/IkappaBalpha will be determined by x-ray crystallography. Structure determination and analysis together with binding and ubiquitinylation assay data will reveal one mechanism employed by nature to disrupt the ability of NF-kappaB to activate expression of new HIV. It will serve as a template for future studies aimed at small molecule intervention of this process with the aim of rendering latent reservoirs of HIV incapable of reproducing.

Results: Under our reaction conditions, we do not detect an interaction between recombinant Murr1 and NF-kappaB, either alone or in complex with IkappaBalpha. We are investigating the structure of the native and recombinant Murr1 proteins to account for the difference between the observations made in cell-based assays and our reductionist in vitro biochemical system. Recent reports indicate that Murr1 is a member of a family of conserved genes that share a structural motif that enables them to interact with NF-kappaB. We are currently focused on identifying the basis of this structural motif so that we can further pursue understanding the effect of Murr1 on NF-kappaB-dependent HIV viral transcription.

Latency and Viral Mechanisms

HIV-1 Tat Superinduces NF-kB Responsive Gene Expression through Inhibition of the SIRT1 Deacetylase

Presenter: Hye-Sook Kwon, J. David Gladstone Institutes, San Francisco

Collaborator: Melanie Ott

Principal Investigator: Hye-Sook Kwon

UARP Award Number: F05-GI-203

The viral Tat protein transactivates the HIV-1 promoter through binding to the TAR RNA element. Tat also modulates the expression of cellular genes in a TAR-independent manner. Here, we report that Tat increases the activity of the transcription factor NF-kappa B by interfering with the nicotinamide adenine dinucleotide-dependent deacetylase SIRT1. Tat directly interacts with the deacetylase domain of SIRT1 and blocks the ability of SIRT1 to deacetylate lysine 310 (K310) in recombinant NF-kappa B/p65. Consistent with these findings, we show that Tat expression induces hyperacetylation of cellular p65 in the presence of SIRT1 using antibodies specific for acetylated K310 in p65. In cotransfection experiments using NF-kappa B reporter genes, Tat neutralizes the negative effect of SIRT1 on the transcriptional activity of wildtype p65, but not of mutant p65 in which lysine 310 was changed to arginine (K310R). This neutralizing effect is lost on a mutant SIRT1 protein that no longer binds to Tat. Tat expressed in mouse embryonic fibroblasts (MEFs) isolated from SIRT1-/- mice has no effect on the activity of endogenous NF-kappa B responsive genes as determined by real-time PCR. In contrast, Tat expressed in MEF cells in which SIRT1 expression has been reconstituted after retroviral infection superinduces transcriptional activities of endogenous I kappa B alpha and E-selectin genes in response to TNF alpha. These experiments collectively show that Tat activates NF-kappa B responsive gene expression through SIRT1, a TAR-independent mechanism that can contribute to Tat-mediated superinduction of the HIV promoter as well as cellular promoters in activated CD4+ T cells.

Latency and Viral Mechanisms

An In Vitro Model of HIV Latency in Primary CD4 Cells

Presenter: Celsa Spina, University of California, San Diego

Collaborators: Paula Campos-Soto, Valeri Terry

Principal Investigator: Celsa Spina

UARP Award Number: ID03-VASD-033

Background: Current treatment of HIV infection does not lead to viral eradication or complete suppression of viral replication. HIV persists in various cell reservoirs; viral replication continues at some level and can be accelerated during in vivo episodes of immune activation. Latently-infected, quiescent CD4 lymphocytes represent an important, highly stable viral reservoir (estimated half life of 6-44 months), and thus present a major barrier to eradication of HIV infection. Studies of latently-infected CD4 cells, and the potential effects of new therapies on this nonproductive viral state, are difficult to perform in vivo, due to the very low frequency of such infected cells in the peripheral circulation (1-10 per million CD4 lymphocytes). Before new treatment strategies can be rationally designed and targeted to latently-infected cell reservoirs, we must first understand the basic biologic mechanisms underlying the establishment and maintenance of persistent, nonproductive HIV infection in CD4 lymphocytes. To address the difficulties existing currently in this research area, we are using a well-defined, primary T cell model to elucidate the viral and host cell variables that interact to develop and maintain a persistent/latent HIV infection in quiescent CD4 cells.

Method: In this model, primary CD4 cells are isolated from healthy HIV-seronegative donors by negative selection, infected with the NL4-3 clone of HIV-1, and stimulated by immobilized anti-CD3 plus anti-CD28 antibodies. Cultures are maintained, in the presence of selected cytokines (IL-2, IL-15, IFN-beta), for 14 to 20 days. After several division cycles, cells return to a predominantly resting state by day 14; and HIV survives in an integrated state within these cells. Sequential analyses are done for: cell activation and proliferation, soluble p24 production, and copies of integrated HIV DNA.

Results: To examine whether the resultant pool size of latently infected cells is dependent on the stage of cell activation that exists when a T cell encounters infectious HIV, CD4 cells were infected at different time points following activation, and the number of cells with persistent viral infection (integrated HIV DNA) after 2 weeks in culture were quantified. Results from these experiments showed that HIV infection in the time period 24 hrs. prior to cell stimulation is optimal for both active virus replication and the development of maximal numbers of surviving T cells that harbor latent HIV. In a second series of experiments, we addressed whether acutely infected cells can survive rounds of cell division to become persistently infected. Cells were initially stained with CFSE viable dye to track cell proliferation; and antiretroviral drugs were added 4 days following cell stimulation, to prevent further rounds of infection. At the end of culture, the infected cells were FACS-sorted based on their proliferation profile (CFSE content). The recovered cell subsets were analyzed for integrated HIV DNA copy number; and parallel aliquots were subjected to a second round of cell stimulation to test the replication competence of any detected HIV integrants. These results demonstrated that fully activated CD4 lymphocytes can survive productive HIV infection to become quiescent cells that carry latent/persistent virus infection.

Conclusion: This long-term culture system provides a highly relevant biological model for the study of viral-host cell interactions that occur during the establishment and maintenance of persistent HIV infection in CD4 lymphocytes. Our cell model also has the potential to aid preclinical studies in the optimization of antiviral therapy strategies.

Latency and Viral Mechanisms

Mechanism of HIV Post-Intergration Latency: Role of MURR-1 Protein and NFAT Transcription Factors

Presenter: Carmen Martin Ruiz-Jarabo, J. David Gladstone Institutes, San Francisco

Collaborator: Warner C. Greene

Principal Investigator: Carmen Martin Ruiz-Jarabo

UARP Award Number: F05-GI-207

Background: The ability of HIV to establish post-integration latency in memory CD4-T resting cells appears to play a key role in the rebound observed after the withdrawal of anti-retroviral therapy in HIV patients, and the persistence of the infection. One approach to the elimination of this latent reservoir is to activate the latent viral genome thus making the virus susceptible to anti-retroviral drugs. However, the initial attempts to deplete the latent reservoirs have failed due in part, to our limited understanding of the molecular mechanisms underlying HIV latency. Studies on the different proteins involved in transcriptional activation of the HIV latent proviruses are pivotal. In our laboratory we are interested in the study of two of these proteins: the NFAT family and the NF-kB/Rel family of transcription factors. Recently our laboratory found that the members of the NF-kB/Rel family play key roles in both maintenance and loss of HIV latency. Specifically, the binding of NF-kB/p50 homodimers to the HIV LTR reinforces viral latency through their function as transcriptional repressors and their recruitment of histone deacetylase-1 complexes. Conversely, transcriptionally active forms of NF-kB promote activation of the latent HIV LTR (Williams et al. 2005 EMBO J. In press). Following these initial studies, we are interested in the contribution of the Murr1 protein (recently identified as an inhibitor of both basal and stimulus coupled induction of p50/RelA heterodimers) to HIV latency. We will also analyze the contribution of NFAT proteins to the activation and/or repression of transcription of HIV latent proviruses in a Jurkat T-cell model of post-integration HIV latency (J-Lat cells).

Methods: (1) To analyze the effect of Murr1 depletion and overexpression in J-Lat cells and in primary latently infected T cells from HIV infected patients. (2) To analyze the effect of NFAT proteins depletion and overexpression in the J-Lat model. To analyze the kinetics of occupancy of the LTR promoter by NFAT and NF-kB proteins in this same model using chromatin immunoprecipitation assays after different stimuli.

Results/Expected Results: Preliminary results in our Jurkat T-cell model of post-integration HIV latency show no alteration in HIV expression after depletion or overexpression of Murr1. However, stimulation of calcium dependent pathways or overexpression of NFAT1 and NFAT2 proteins in this same model promoted the activation of the latent provirus, suggesting an important role for these proteins in transcriptional activation.

Conclusions: The precise cellular factors and intracellular events that underlie the establishment and maintenance of HIV latency remain poorly defined and may in fact be multifactorial. Understanding how the latent state is both maintained and forfeited will provide important insights into the molecular basis of HIV latency and could suggest new approaches to purging the virus from the latent reservoir

Latency and Viral Mechanisms

HIV-1 Tat Upregulates CXCR4 and Increases Chemotaxis to SDF-1

Presenter: Prerana Jayakumar, J. David Gladstone Institutes, San Francisco

Collaborator: Eric Verdin

Principal Investigator: Prerana Jayakumar

UARP Award Number: D04-GI-410

The HIV-1 Tat protein is a transcriptional activator of the HIV promoter and is also thought to be involved in the activation of immune T cells. We have investigated the mechanism of Tat action by examining the expression of 21,000 human genes using oligonucleotide microarray analysis in response to endogenous Tat expression. We compared two forms of Tat, Tat72 and Tat101 with control cells. Among the genes identified is the chemokine receptor, CXCR4. We found that both Tat72 and Tat101 induce CXCR4 at the cell surface. Furthermore, this increase in CXCR4 expression is paralleled by an increase in the chemotaxis of cells that express Tat to the ligand of CXCR4, SDF-1. Both Tat72 and Tat101 also activate the CXCR4 promoter. The results may implicate Tat in altering cellular homing to sites of infection as well as signaling through chemokine receptors.

Vaccine Development & Immunology—I

Fc gamma RIIa Genotype Influences the Relationship between Antibody Titer and Infection Risk among Vaccinees in the VaxGen 004 Trial

Presenter: Donald Forthal, University of California, Irvine

Collaborators: Peter Gilbert, Gary Landucci, Tran Phan, Renee Higa-Tanner

Principal Investigator: Donald Forthal

UARP Award Number: ID04-I-020

Background: Results from the VaxGen 004 trial indicated that vaccination with recombinant gp120 (rgp120) was ineffective in preventing sexually transmitted HIV infection. However, vaccine induced antibody titers correlated inversely with HIV infection risk. Since antibody function is often highly dependent on interactions between the Fc part of antibody and Fc gamma receptors (FcgRs) found on monocytes, macrophages, dendritic cells, and natural killer cells, we sought to determine if a functional polymorphisms in an FcgR gene might influence the relationship between antibody titer and infection risk.

Methods: Allele-specific primers were used to determine the FcgRIIa (histidine [H] vs. arginine [R]) genotypes in rgp120-vaccinated participants in the VaxGen 004 trial. The sample included 221 of the total of 241 vaccinated participants that became infected during the study and 138 randomly selected uninfected vaccinees. 50% neutralizing antibody titers against HIVMN have been reported previously and were measured by determining the dilution of serum that blocked HIV-1-induced cytopathic effect on MT4 cells.

Results: There was an inverse correlation between HIV risk and HIVMN neutralizing antibody titer among individuals with RR and RH genotypes, but not among those with the HH genotype:

Genotype	*RelRisk per log10 titer increase*	*p-value*
RR	0.54	0.023
RH	0.39	0.021
HH	1.49	0.35

Furthermore, the relative risk of infection for vaccinees with the HH genotype compared to those with the RR genotype increased with increasing antibody titers (relative risks = 0.34, 0.74, 0.90, and 1.64 for antibody titer quartiles 1, 2, 3, and 4, respectively).

Conclusions: These results suggest that the FcgRIIa polymorphism influences the previously reported inverse correlation between vaccine-induced antibody responses and risk of HIV infection among participants in the VaxGen 004 trial. Specifically, individuals with the HH genotype have a non-statistically significant increased risk of infection with increasing antibody titers, whereas individuals with the RH or RR genotype have a lower risk of infection with higher antibody titers. The HH receptor is known to have higher affinity for IgG2- and IgG3-immune complexes than do the RH or RR isoforms. Thus, our findings might be explained by FcgRIIa-mediated antibody-dependent enhancement (ADE) of infection by infectious immune complexes that is most likely to occur in the presence of the higher-affinity receptor isoform. This would in turn suggest that potentially beneficial antibody or innate immune functions that might operate in individuals with RH or RR genotypes are partially overcome by ADE in HH homozygotes. Our data are consistent with the recent observation that infants who are homozygous for the H allele are more likely to acquire HIV perinatally from their infected mothers than infants with other FcgRIIa genotypes.

Vaccine Development & Immunology—I

Multimeric Soluble CD40L or GITRL Are Molecular Adjuvants for HIV DNA Vaccines

Presenter: Richard Kornbluth, University of California, San Diego

Collaborators: Geoffrey W. Stone, Suzanne Barzee, Victoria Snarsky, Kristin Kee, Xiao-Fang Yu, Celsa A. Spina

Principal Investigator: Richard Kornbluth

UARP Award Number: ID04-VMRF-031

Background: CD40 ligand (CD40L, CD154), a member of the TNF superfamily (TNFSF), is the major endogenous activator of dendritic cells, enabling them to "license" CD8+ T cells for long-term memory. CD40L is a trimeric, membrane molecule that has been very difficult to apply to vaccination. However, we have constructed new forms of CD40L that are soluble proteins with 1-, 2-, or 4-trimers. Previous work indicated that the immunostimulatory activity of the soluble trimers is related to the valency of the trimers ($1 < 2 < 4$). In addition, a 4-trimer form of GITRL, another TNFSF molecule, was prepared for study. GITRL is of interest because it reverses the suppressive effects of CD4+CD25+ regulatory T cells (Tregs) and co-stimulates CD4+CD25- effector T cells. We wished to determine the effect of soluble, multimeric CD40L and GITRL on long term immunological memory, either 14 or 90 days after vaccination with a secreted Gag DNA vaccine.

Methods: Antigen plasmid was pScGag, expressing a secreted form of the Gag protein from a CMV promoter. The following TNFSF plasmids were tested: pMemCD40L (natural membrane CD40L); pAcrp30-CD40L (2-trimer sCD40L produced as a fusion protein with Acrp30); pSP-D-CD40L (4-trimer sCD40L produced as a fusion protein with surfactant protein D); and pSP-D-GITRL (4-trimer sGITRL). BALB/c mice were vaccinated i.m. with 80 ug of antigen plasmid plus 20 ug of adjuvant plasmid every two weeks X 3, and spleen cells were harvested either 14 or 90 days later.

Results: Plasmids for the natural membrane form of CD40L (pMemCD40L) had no adjuvant effects on the formation of tetramer positive CD8+ cells. However, 4-trimer sCD40L (pSP-D-CD40L) increased the level of tetramer positive CD8+ cells both 14 and 90 days post vaccination. At 90 days post vaccination, this adjuvant effect was seen for both CD62L low (effector memory) and CD62L high (central memory) pools. pSP-D-GITRL also augmented the level of tetramer positive CD8+ cells, but led to a predominance of CD62L high central memory cells. Conclusions: The addition of pSP-D-CD40L to secreted Gag DNA vaccines produced CD8+ T cell memory responses that survive for more than 90 days after vaccination. A pSP-D-GITRL adjuvant also produced tetramer positive CD8+ T cell responses, and favor the generation of CD62L high central memory cells. Taken together, these soluble multimeric TNFSF ligands have significant potential as DNA vaccine adjuvants.

Vaccine Development & Immunology—I

Role of Glycopeptides in HIV-specific CTL generation

Presenter: Alessandra Franco, University of California, San Diego

Principal Investigator: Alessandra Franco

UARP Award Number: ID04-SD-021A

Glycopeptides having high affinity for MHC class I molecules have been found to generate short-term and memory anti-carbohydrate specific cytotoxic T lymphocytes (CTLs) to tumor associated carbohydrate antigens (TACA) in mice, which did not require T cell help (Th). Since long-term infection with human immunodeficiency virus (HIV) results in depletion of CD4 helper T cells, the stimulation of a similar helper-independent CTL response would be of great value for the development of vaccine strategy against HIV. Furthermore, glycan-modified HIV determinants, tested for their ability to prime in vitro peripheral blood mononuclear cells (PBMC) derived from normal donors, result in superagonistic antigens capable of inducing a large CTL repertoire with very high affinity. The potential higher degeneracy of CD8+ T cells generated by glycopeptides relative to peptides has a great advantage in strategies aimed at controlling viral infection with such diversity as HIV-1. The study here proposed will explore a novel CTL-based vaccine approach that uses GlcNAc-modified HLA-A2 conserved viral sequences as immunogens to prevent/treat HIV-1 infection. Based on the high level of glycosylation found in HIV-1 envelope glycoproteins and the successful generation of degenerate anti-carbohydrate-specific CTLs to tumor antigens, HIV-derived glycopeptides are anticipated to generate a high affinity, T helper-independent and highly cross-reactive CTL repertoire. This study may indicate a low-cost and innovative strategy to improve currently available vaccines, addressing the critical problem of viral mutations.

Vaccine Development & Immunology—I

The Role of Nef-mu1 Interactions in the Modulation of MHC I

Presenter: Colleen Noviello, Veterans Medical Research Foundation, San Diego

Collaborator: John C. Guatelli

Principal Investigator: Colleen Noviello

UARP Award Number: D05-VMRF-404

Background: HIV-1 evades cellular adaptive immunity by Nef-mediated removal of class I major histocompatibility complex (MHC I) from the cell surface. Nef binds the clathrin adaptor protein-1 complex (AP-1), both through the hemi-complex of gamma-sigma subunits and mu1 subunit alone. While the gamma-sigma hemi-complex interaction is dependent upon the acidic-dileucine motif found in the unstructured C-terminus of Nef, the mu1 interaction is independent of this motif. Recently, a ternary interaction has been revealed between the AP-1 complex, Nef, and the MHC I molecule itself. Since the dileucine motif is not required for the modulation of MHC I, we propose that the Nef-mediated interaction with the mu1 subunit of AP-1 is responsible for this effect. This idea is strengthened by the existence of a putative "cryptic" tyrosine-based sorting signal in the cytoplasmic tail of MHC I; the tyrosine in this sequence is required for the Nef effect, and similar tyrosine-based signals in cellular proteins bind to the mu subunits of AP complexes. Thus, we hypothesized that the MHC I cytoplasmic tail binds to the ? subunit of AP-1 in the presence of Nef, which binds to both mu1 and the MHC I tail.

Methods: We tested this hypothesis by measuring protein-protein interactions using yeast two- and three-hybrid assays. We also utilized the GST-pulldown system, expressing GST-tagged proteins in E.coli to use as the "bait", and using HeLa cell lysate as the source for cellular proteins.

Results: We have not detected an interaction between the cytoplasmic tail of MHC I and Nef or between the tail and mu1 in the yeast two-hybrid system. We have also not detected a ternary interaction between the MHC-I tail, Nef and mu1 using the three-hybrid system. However, we observed that the MHC-I tail, when fused to Nef, increases the efficiency of interaction with intact AP-1 as measured in GST pull-down experiments. Interestingly, the MHC-1 tail does not render the interaction between Nef and AP-1 independent of the Nef dileucine motif. We are currently determining whether the contribution of the MHC-I tail to the interaction with AP-1 requires the tyrosine residue necessary for the modulation of MHC-I by Nef.

Conclusions: We will use the MHC-I tail/Nef chimera in our yeast two- and three-hybrid assays to determine whether the contribution of the tail to the interaction with AP-1 is due to an increased interaction with the mu1 subunit or with the hemi-complex of gamma-sigma subunits. These data should allow us to elaborate a more precise model for the ternary interaction between MHC-I, Nef, and AP-1.

Vaccine Development & Immunology—I

Expression of SIV Genes in a Simian Varicella Zoster Virus Vaccine

Presenter: Lawrence Feldman, University of California, Los Angeles

Principal Investigator: Lawrence Feldman

UARP Award Number: ID04-LA-018

Background: This project was ultimately designed to develop a vaccine for HIV using a human herpesvirus, Varicella-Zoster Virus, as the vector. Factors that make Varicella an attractive candidate for a vector include the following properties. It is a large DNA virus. It is an enveloped virus that is capable of infecting epithelial cells, mucosal cells, and T cells hematogenously. Because it is a large DNA virus, it is capable of encoding multiple HIV genes. Finally, it is a safe vector that is already FDA-approved for use in the U.S.

Methods: As a proof of principle, we took two analogous steps back, so that we could use an animal model. For a vector, we decided to use Simian Varicella Virus (SVV). This is the primate equivalent of Human Varicella Zoster Virus, and shares with it many of the same structural and functional properties. For a virus that could infect primates, we chose SIV, as the primate analogous virus to HIV. Based on similarity of function between SVV and VZV, there are several genes within SVV that could be deleted without harming its ability to replicate in vitro.

A technical difficulty in this study is that SVV is not able to form plaques with cell-free virus. The virus that egresses from the cell is not infectious. To overcome this difficulty, researchers of Varicella and SVV have used a genetic system of overlapping cosmids which are linearized and cotransfected into permissive cells. For SVV, these cosmids are approximately 45 kb in size. SVV DNA is not cut with the restriction enzyme Not I; the cosmid clones are constructed with Not I ends. To make infectious virus, four overlapping DNA cosmids are restricted with Not I, purified, and co-transfected into Vero cells.

Another technical problem in this field is that cosmid clones are so large that making a recombinant virus often involves an elaborate series of cloning steps. In our laboratory we have been able to use single copy cosmid vectors, called fosmids, for making viral mutants of varicella. For this project we tried to clone the 124kb of SVV into several fosmid clones. After several months of partial success, we decided to obtain a set of infectious cosmid clones from Wayne Gray.

Results: Most of the work has involved constructing a fosmid vector that will harbor Dr. Gray's clones but at one copy per cell, and moving these large DNAs into that vector. At present we have moved all four of the vectors, but the four DNAs together are not infectious.

Conclusions: Once infectious clones are obtained, we will insert into one of the fosmids a set of SIV genes. We have obtained a retanef clone from Dr. G. Franchini. The SIV retanef clone is a chimeric polyprotein composed of open reading frames for rev, tat and nef (G. Franchini, Vaccine 20 (2000) 3171-3186. Our plan is to clone these genes into the SVV virus between open reading frames 13 and 14. Orf 13 and Orf 14 lay in opposite directions on the viral genome, and neither is essential for viral replication. However, the plan is to put the retanef genes between the poly A of Orf 13 and the promoter of Orf 14, without deleting any SVV sequences.

Vaccine Development & Immunology—I

HIV-Induced Bystander Cell Death in Human Lymphoid Histocultures

Presenter: Gilad Doitsh, J. David Gladstone Institutes, San Francisco

Principal Investigator: Gilad Doitsh

UARP Award Number: F04-GIVI-210

Background: HIV-1 infection leads to the progressive loss of CD4 T cells eventually culminating in the clinical presentation of AIDS. Despite more than 20 years of study, the mechanism underlying the progressive CD4 T-cell depletion remains poorly understood. Specifically, the relative importance of direct cytopathic effects of the virus versus indirect death of uninfected bystander cells remains a key question. Recent studies indicate that most dying CD4 T cells in the peripheral blood and lymph nodes of HIV-infected patients are un-infected, suggesting that bystander killing plays a major role in AIDS pathogenesis. The molecular basis for bystander killing has not been completely defined and is difficult to address in in-vivo studies.

Methods: In our experiments, we have utilized an ex vivo human lymphoid culture system involving primary human tonsil tissue, which recapitulates the cellular complexity of lymphoid organs where HIV infection principally occurs. Furthermore, HIV infection in these tissues does not require the addition of mitogenic stimuli or cytokines. As such, this ex vivo culture system forms a powerful experimental approach to modeling the molecular and cellular events occurring during HIV infection in vivo.

Results: Using this system, we observe massive loss of uninfected bystander CD4 T cells after infection with CXCR4-tropic viruses. This bystander killing critically depends on interaction of the viral envelope glycoprotein gp120 and the chemokine receptor CXCR4 on the surface of CD4 T cells. Interestingly, HIV virions alone induce a much lower level of bystander killing than that found when uninfected and HIV-1-infected tonsil cells are mixed together. These findings coupled with Boyden chamber experiments argue for an important role of cell-cell interaction. Of note, bystander killing is particularly prounounced when activated CD25+ CD4 T cells are employed as uninfected target cells.

Conclusions: The fact that cell-cell interactions are required for bystander killing suggests that gp120-CXCR4 interaction alone cannot induce the killing response and additional interactions between uninfected and infected CD4 T cells play a role in this process. It is also possible that further interactions with neighboring B cells or CD8 T cells are required. The potential role of these cell-cell interactions is under active investigation. A clear understanding of the molecular basis for bystander cell killing in HIV infection might propel new therapeutic strategies aimed at blocking these interactions and reducing CD4 T-cell depletion. Such approaches might markedly delay the progression to AIDS in HIV-infected patients.

Vaccine Development & Immunology—I

Structural Biology of Chemokines and Their Receptors

Presenter: Tracy Handel, University of California, San Diego

Principal Investigator: Tracy Handel

UARP Award Number: ID03-SD-005

Background: Chemokines are well recognized as the traffic signals for cell migration in inflammation, routine immune surveillance, and lymphocyte development and homing. They function by binding to specific GPCRs on many types of cells, causing conformational changes that trigger cascades of intracellular signaling pathways involved in cell movement and activation. Although chemokines have evolved to carry out developmental and protective roles, inappropriate expression or utilization of these proteins is associated with a broad spectrum of diseases ranging from cancer metastasis, atherosclerosis, and inflammatory disease, to global life-threatening disease like malaria and AIDs. Nevertheless, relatively little is known about their structural and functional properties, information which is critical for the discovery of strategies to interfere with their function and for the development of molecules with therapeutic potential. To aid such endeavors, we have been using structural and biochemical methods to understand the molecular details of chemokine-receptor interactions and function. In particular, we have been investigating the structural details of how chemokines interact with chemokine receptors, glycosaminoglycans and viral chemokine binding proteins.

Methods and Results: We have shown that (i) chemokines bind receptors as monomers, (ii) we identified receptor binding, signaling and glycosaminoglycan (GAG) binding hotspots on several ligands, (iii) we showed how receptor binding and signaling could be decoupled, thereby creating antagonists, (iv) using engineered mutants, we demonstrated that GAG-binding and oligomerization of chemokines are critical for their function in vivo, and (v) we demonstrated that GAG-binding and oligomerization are functionally coupled, and proposed the concept that different oligomerization states may add to the specificity of GAG-binding, with important biological consequences like cellular localization. From these types of detailed studies, several strategies to antagonize chemokine function have become apparent: chemokine mutants that bind but do not activate their receptor, GAG-binding deficient mutants, oligomerization deficient mutants, and GAGs themselves.

Our most recent work has involved strategies for producing high levels of functional reconstituted receptors for a wide range of biophysical studies. To this end we have pursued several types of expression systems including an inducible system in mammalian cells. Thus far we have two receptors expressed at high level, and are currently working on determining requirements for functional reconstitution. Once established, we plan to investigate interactions with their ligands, and lead compounds using NMR, EPR, mass spectroscopy and ultimately crystallography.

HIV Clinical Research Topics and NeuroAIDS

Rate of Viral Evolution and Risk of Losing Future Drug Options in Heavily Pre-treated Patients Remaining on a Stable Partially Suppressive Regimen

Presenter: Hiroyu Hatano, University of California, San Francisco

Collaborators: Peter Hunt, Jodi Wiedler, Eoin Coakley, Rebecca Hoh, Teri Liegler, Jeffrey Martin

Principal Investigator: Steven Deeks

UARP Award Number: ID04-SF-012

Background: Many treatment-experienced patients with limited therapeutic options for complete viral suppression remain on a partially suppressive regimen pending the availability of at least 2 fully effective agents. The major risk of this approach is ongoing viral evolution and the loss of future drug options. The rate at which drug options are lost in such patients has not been carefully defined.

Methods: Antiretroviral-treated patients with drug resistance were sampled from a clinic-based cohort of chronically HIV-infected patients. Subjects were included in this analysis if they had: stable antiretroviral regimen for ³120 days, plasma HIV RNA level >1000 copies/mL, at least 1 genotypic resistance mutation, and at least 1 follow-up visit. Phenotypic and genotypic resistance testing was performed every 4 months and observations were censored at the time of any treatment modification. The primary endpoints were time to loss of phenotypic susceptibility to at least 1 fully effective drug (or 2 partially effective drugs), as well as time to development of 1 new nucleoside analogue mutation (NAM) or 1 new major protease mutation (IAS-USA guidelines).

Results: A total of 106 patients were eligible; the median duration of observation was 48 weeks (IQR 32-90). The median baseline CD4 cell count and plasma HIV RNA levels were 292 cells/mm3 and 3.74 log copies/mL, respectively. Subjects had previously received a median 8 prior drugs. Using a Kaplan-Meier analysis, the estimated risk of losing 1 fully suppressive drug (or 2 partially suppressive drugs) was 32% at 1 year (95% CI 22-45). The risk of developing a new NAM at 1 year was 23% (CI 12-40), and of developing a new major protease mutation was 17% (CI 8-33). We also assessed the risk of deferring the use of a single new drug (tipranavir). Only an estimated 5% (CI 2-16) of protease inhibitor-treated patients experienced genotypic loss of tipranavir susceptibility. Viral load, CD4+ T cell counts, phenotypic susceptibility score, replicative capacity, number of previous drugs, and number of missed doses in last 30 days were not consistently predictive of rate of viral evolution.

Conclusion: In a cohort of heavily pretreated HIV patients with incomplete viral suppression, the risk of losing future drugs options appeared to be moderate. This risk should be considered when deciding to maintain patients on a partially suppressive regimen.

HIV Clinical Research Topics and NeuroAIDS

In Vivo Astrocyte Metabolism Using Acetate-2-^{13}C MRS in HIV

Presenter: Brian Schweinsburg, University of California, San Diego

Principal Investigator: Brian Schweinsburg

UARP Award Number: ID05-SD-038

Background: Human immunodeficiency virus (HIV) is associated with brain injury. Previous studies have demonstrated that HIV infection is related to reductions in brain volume, alterations in brain chemicals that are sensitive to cellular damage, and neurocognitive dysfunction. However, the underlying mechanisms of damage are not well understood, particularly in individuals living with HIV. In cellular models of HIV infection, it is hypothesized that alterations in certain neurotransmitter systems may be responsible for neural injury. One such system is the major excitatory neurotransmitter glutamate. HIV affects glutamate neurotransmission through a number of potential mechanisms. As an example, HIV may alter the release and recycling of this amino acid. Neurotransmitter glutamate is released by neurons and subsequently brought into neighboring cells called astrocytes where it is converted into glutamine. Astrocytes perform this vital function, in part, to "detoxify" glutamate before sending it back to the neuron. Excess glutamate surrounding neural cells can be toxic and can eventually result in neuronal death. This dysregulation is called glutamate-related excitotoxicity. While this is a likely mechanism of brain injury in HIV disease, no study has directly assessed the impact of HIV on glutamate/glutamine recycling in the living human brain.

Methods: Carbon-13 (^{13}C) magnetic resonance spectroscopy permits in vivo measurements of this important metabolic process. To help track metabolism, a non-radioactive, ^{13}C-labeled substrate is infused and the ^{13}C label is passed through important metabolic cycles, including the glutamate/glutamine cycle, which reflects glutamate neurotransmission. The advantage of this technique is that one can non-invasively monitor the complete metabolism of the infused substrate, generating valuable metabolic information. In this study, ^{13}C MR spectroscopy will be performed during an intravenous infusion of sodium-acetate-2-^{13}C to study the effects of HIV on astrocyte metabolism. Eight HIV+ and 8 HIV seronegative healthy controls (HIV-) will be enrolled.

Expected Results: We predict that HIV+ participants will display increased rates of sodium acetate-2-^{13}C label incorporation into C4-glutamate relative to healthy, HIV seronegative control participants (HIV-). This will reflect an increase in the astrocyte tricarboxylic acid (TCA) cycle rate, VtcaA. In addition, we predict that HIV+ participants will display decreased rates of sodium acetate-2-^{13}C label incorporation into C4-glutamine relative to healthy, HIV seronegative control participants (HIV-). This will reflect a decrease in glutamate-glutamine cycling, Vcycle, with the result that less glutamate is recycled to glutamine, leading to potential excitotoxicity.

Conclusions and Future Directions: The project is innovative in that it will be the first to measure glutamate neurotransmission in individuals living with HIV. Our future objectives are to use this technique to characterize the mechanisms of HIV-related brain disease and monitor the effectiveness of antiretroviral therapy. The ability to monitor astrocyte metabolism and glutamate neurotransmission has particular relevance for identifying HIV-related neural mechanisms of damage and monitoring the outcome of treatment in the living brain where the ultimate goal is to improve the quality of life and reduce the healthcare burden of Californians living with HIV. Furthermore, by elucidating important aspects of the excitotoxic cascade, it may be possible to advance neuroprotective treatments.

HIV Clinical Research Topics and NeuroAIDS

California Collaborative Treatment Group (CCTG)

Presenter: Eric Daar, Harbor-UCLA Medical Center, David Geffen School of Medicine at UCLA

Collaborators: Eric Daar, Jeremiah Tilles, Carol Kemper, Robert Larsen

Principal Investigator: Richard Haubrich

UARP Award Number: CH05-SD-607 -005

Since 1986, the California Collaborative Treatment Group (CCTG) has been conducting high impact multi-centered, investigator- initiated clinical trials that address the primary theme of our application: Emerging Problems in the Management of HIV Infection. The group has over 140 publications and has a proven record of accomplishments that include: providing access to research opportunities for underrepresented populations, addressing research questions of importance to HIV infected patients in California, collaborating with diverse disciplines to expand the scope and depth of our research projects, mentoring junior investigators to become the next generation of California clinical investigators, and leveraging funding from additional sources, to build on core funding from the UARP.

The CCTG is a five- center, academic affiliated clinical trials group. The CCTG agenda is facilitated by monthly meetings, where we present new concepts, work on existing protocols and discuss new data. Junior investigators are encouraged to lead these discussions. The CCTG agenda encompasses three major studies:

CCTG 584- Viral dynamics and pharmacokinetics of tenofovir (TDF) and abacavir (ABC). The goal of this study is to determine the pathogenesis of the poor virologic response to TDF + ABC containing regimens. By studying the interaction between TDF and ABC, we can explore the mechanism and determine the viability of this combination. The hypothesis is that the dual NRTI combination will be less potent than either drug used alone and that the difference can be explained in part by an intracellular interaction (reduced levels of intracellular active phosphoralated compounds). The specific primary aims are: to evaluate the relative potencies of TDF or ABC given alone for 7 days compared to TDF + ABC as assessed by the short-term HIV RNA response; and to compare the plasma and intracellular pharmacokinetic data of monotherapy vs. the dual NRTI regimen.

CCTG 585- A comparison of once daily Lopinavir/ritonavir (LPV/r) given as liquid versus capsules. The goal of this study is to determine if we can simplify antiretroviral therapy with LPV by evaluating two once daily LPV regimens. Although LPV has been shown to be effective in once daily dosing, diarrhea limits its utility. The hypotheses for this study is that once daily LPV/r liquid will be better tolerated than once daily LPV/r capsules. The primary objective of the study is to compare the tolerability of once daily LPV/r (800/200 mg) given as 10 ml liquid vs. 6 capsules.

CCTG 587- Pathogenesis of community-acquired MRSA among HIV+ MSM. An important emerging new problem for HIV infected patients is infection with methicillin resistant Staphylococcus aureus. Understanding the epidemiology, risk factors and host defenses of this infection will be important to designing strategies to treat and prevent the infections. The objectives of this study are: 1) to prospectively determine the prevalence, incidence, persistence, and risk factors for asymptomatic MRSA colonization among HIV infected MSM and control groups; 2) to compare colonizing MRSA strains with MRSA strains causing clinical infection and to quantify the persistence of strain colonization; and 3) to identify factors in host defenses and host-defense interactions that may prevent S. aureus infections.

These studies will address research questions of importance to HIV infected patients in California and will allow the CCTG to continue with our ancillary missions of providing research access to minority patients, mentoring junior investigators and expanding collaborations with diverse disciplines.

HIV Clinical Research Topics and NeuroAIDS

1- to 3-Year Outcomes in HIV-infected Liver and Kidney Transplant Recipients

Presenter: Michelle Roland, University of California, San Francisco

Principal Investigator: Peter Stock

UARP Award Number: TP00-SF-154

Background: Organ failure is a significant problem for patients with HIV infection in the current era of HAART). Improvements in HIV-associated morbidity and mortality have made it difficult to continue denying solid organ transplantation to this population based upon futility arguments alone. Despite the need for transplantation, the safety and efficacy of this intervention in HIV-infected recipients is unknown. In this study, we describe the patient, graft and HIV-specific outcomes of the largest prospective cohort of kidney and liver recipients followed for 2 to 5 years in the HAART era.

Methods: Prospective, multi-site cohort of HIV-infected transplant recipients followed for patient and graft survival, HIV outcomes (opportunistic complications [OI], CD4+ T-cell count, HIV RNA) and rejection. Subjects had CD4+ T-cell count > 100/200 (L/K) and undetectable HIV RNA or prediction of full suppression (L). Time to subject and graft failure and cumulative rejection were analyzed using the Kaplan-Meier technique.

Results: 29 subjects received transplants (18 K/11 L) from 3/00 - 11/03. 28 were male; median age was 45 (15-64) yrs. 16 were White, 10 African American, 2 Asian and 1 Hispanic. Five had a history of OI (KS, CMV, PCP, cryptococcal meningitis, TB). 2 L subjects had detectable pre-transplant HIV RNA. 6 L and 5 K subjects had hepatitis C. There were 2 deaths at 7 and 15 mos. from recurrent HCV in L subjects and 1 at 6 months from congestive heart failure in a K subject. The estimated 1-year survival rates (SE) were 94% (6) for K and 91% (9) for L; the 3-year rate decreased to 81% (13) in L. There were 2 K graft losses at 8 days from rejection or vascular thrombosis. The K graft survival estimate, censoring death with graft function, was 89% (7). There was one L graft loss at 7 weeks due to a small-for-size graft lesion. The 1-year L graft survival estimate, censoring death with graft function, was 91% (12). There was 1 case each of CMV (L) and candida (K) esophagitis and 1 anal carcinoma (L); none were in subjects with OI history. The last HIV RNA was undetectable in all but 1 surviving subject. 12 K subjects had one or more rejection; 1-, 2-, and 3-year cumulative K rejection estimates are 52%, 66% and 77% (12%).

Discussion: Good patient and graft survival, stable CD4+ T-cell and HIV RNA levels, and few HIV-associated complications in a select group of HIV-infected subjects suggest that L and K transplant is safe and effective in this population. Data from an additional year of follow-up are currently being analyzed. A larger study will compare outcomes in 275 subjects with HIV-uninfected recipients with special attention to HCV recurrence in L subjects and to elucidating the mechanisms of rejection among K subjects.

HIV Clinical Research Topics and NeuroAIDS

Sex Differences in Lopinavir/Ritonavir Pharmacokinetics among HIV-infected Men and Women

Presenter: Obiamiwe Umeh, University of California, Los Angeles

Principal Investigator: Obiamiwe Umeh

UARP Award Number: CF04-LA-302

Background & Rationale: Early in the HIV epidemic in the USA, HIV/AIDS was a predominately "gay white male" disease. Recently, the prevalence of HIV in women has increased worldwide. Females now make up about half of all persons infected with HIV globally. Historically women have been under-represented in HIV clinical trials. This disparity is most evident in early phase studies of newer agents. Consequently, current knowledge about the pharmacokinetics (PK), efficacy and toxicity of anti-retroviral drugs is derived from studies of predominately male subjects. Data from recent studies focusing on HIV-infected female subjects suggest the existence of sex-related PK differences. While the clinical significance of some of these differences is not fully apparent, several studies suggest that women achieve higher drug levels than men even after controlling for weight. Much of the existing data have important limitations. Some studies were retrospective reviews of therapeutic drug monitoring databases in which the indication for monitoring was not controlled. Other studies were conducted in HIV-uninfected subjects, included random drug levels and often enrolled too few women. Ours is the first large prospective formal PK study of sex differences in Kaletra pharmacokinetics. Kaletra is a co-formulation of lopinavir (LPV) -a potent inhibitor of HIV-1 protease and ritonavir (RTV) which inhibits CYP 3A-mediated metabolism of lopinavir. Kaletra (LPV/r) is widely used to treat HIV infection.

Patients and Methods: *Population*: HIV-infected men and women, who were aged 18 and older on LPV/r in combination with one or more antiretroviral agents for at least 2 weeks prior to screening, were eligible for this study. Subjects on medications interacting with LPV/r, pregnant females and subjects treated with dual HIV protease-inhibitors were excluded.

Study Design: This is a multi-center stratified prospective non-randomized PK study. Females are hypothesized to have a LPV area-under the concentration-time curve (AUC) 30% higher than their male counterparts. A total of 78 subjects were divided into two groups by sex. Each group was further divided into 4 approximately equal strata by race/ethnicity. Three study visits were required; a screening, pre-entry and a PK visit. LPV PK was assessed after subjects kept a Kaletra medication diary for a 48-hour period. One hundred percent compliance with medications during this period was required to enter the study. On the PK day, blood samples were drawn at time 0 and at again at 1, 2, 3,4,5,6,8,10 and 12 hours after subjects took the morning dose of LPV/r. All subjects received a standardized breakfast.

Statistical Analysis Plan: Primary Endpoint: Lopinavir AUC $_{0\text{-}12h}$

Secondary Endpoints: LPV PK parameters maximum concentration (C_{max}), concentration at 12 hours (C_{12h}) and apparent oral clearance (CL/F)

Assumptions: AUC of LPV/r follows a normal distribution. The variance of LPV PK parameters between HIV-infected men and women is equal. A Student's T-test will be used to compare the mean LPV AUC as well as the other PK parameters in the 2 groups. A multiple regression model fitted with stepwise model selection will be used to evaluate the relationship of the covariates age, weight, use of tenofovir and body mass index on the PK of LPV. A non-parametric Kruskall-Wallis test will be performed to evaluate the differences in LPV PK parameters among the racial groups.

Results: This study opened to accrual nationwide on 10/06/05 and rapidly accrued by 11/30/05. Final results are pending.

HIV Clinical Research Topics and NeuroAIDS

Arterial Spin Labeled (ASL) MRI Differentiates HIV Neurocognitive Impairment (HNCI) Subtypes

Presenter: Beau Ances, University of California, San Diego

Principal Investigator: Beau Ances

UARP Award Number: CF05-SD-301

Background: Accurate assessment of cognitive impairment due to HIV in the CNS is critical for determining an individual s neurological prognosis and possible response to highly active anti-retroviral therapy (HAART). Neuronal cell damage as well as reversible alterations in neuronal cell function underlies poor neuropsychological performance (NP). Non-invasive brain imaging provides an objective and quantitative assessment of brain integrity and function.

Methods: Arterial Spin Labeled Magnetic Resonance Imaging (ASL-MRI), was used to determine baseline global and regional cerebral blood flow (CBF) changes in 44 HIV associated neurocognitive impairment (HNCI) patients (24 mild cognitive dementia (MCMD) and 20 HIV associated dementia (HAD)) and 10 seronegative controls.

Results: Baseline global CBF values were similar for all groups. However, baseline CBF within areas known to be affected by HIV, the caudate and globus pallidus, was significantly affected in HNCI patients compared to seronegative controls.

Conclusions: These results suggest that HIV downregulates direct subcortical pathways involved in directed movements. Targeted regional ASL-MRI analysis of CBF within subcortical areas of the brain commonly damaged by HIV may predict individuals at risk for development of cognitive impairment and aid in determining responses to HAART or other neuroprotective therapies.

Vaccine Development & Immunology—II

California Research Center for the Biology of HIV in Minorities

Presenter: Richard Pollard, University of California, Davis

Principal Investigator: Richard Pollard

UARP Award Number: CH05-D-606

Investigators at the University of California, Davis School of Medicine with their partner the Viral and Rickettsial Disease Laboratory of the California Department of Health Services are proposing to form a California Research Center for the Biology of HIV in Minorities. The investigators will also focus on gender differences and will conduct specific studies focused on women. The source of patient subjects for the proposed research will be the Center for AIDS Research Education and Services in Sacramento. The investigators feel that detailed biologic studies focusing on immunologic and virologic differences in HIV-infected subjects of different ethnicities and in women are important to describe differences that can be used to tailor therapeutic interventions depending on the results. The clinical site provides HIV care for a diverse patient population which will facilitate the studies as proposed.

The major research project is focused on studies at local versus systemic HIV-specific immune responses and viral diversity. In order to eventually develop vaccines that are targeted at stimulating local protective responses to HIV exposure, understanding of the presence of such responses and how they change over time will be studied. Studies of viral diversity will also be performed to understand whether or not viruses diverge differently in different sites and they change over time. Viruses will be obtained from genital and rectal sites and compared to those obtained in plasma.

The investigators will accomplish these goals through several infrastructure components. An Administrative Core will manage the finances of the Center, perform regulatory and reporting functions, maintain databases and a website and provide statistical support to the Center. A Pilot Project Core will solicit proposals, conduct their review and monitor their progress. The pilot projects will focus on the overall theme of the Center and be funded by UARP resources as well as additional UC Davis resources. A Clinical Core will develop a repository and clinical database on subjects with specimens in the repository. It will also assist Center investigators in the design and conduct of clinical projects. An Immunology Core will provide advanced flow expertise and instrumentation as well as access to a large number of other immunologic assays. A Virology Core located at the partner institution will perform sequencing, tropism assays, resistance assays and other virologic assays required by the Center investigators.

Vaccine Development & Immunology—II

Aurora B Regulates CD4 T cell Expansion Mediated by CD28 Cosignals

Presenter: Jianxun Song, La Jolla Institute for Allergy and Immunology

Collaborators: Shahram Salek-Ardakani, Wei Duan, Yanfei Adams, Michael Croft

Principal Investigator: Jianxun Song

UARP Award Number: ID05-LJIAI-015

CD28 provides an important costimulatory signal for T cell activation that regulates multiple cellular processes including proliferation, expansion and survival. However, the mechanism by which CD28 regulates these different processes is still not clear. Using MCC-reactive AND transgenic T cells, we demonstrate that after the recognition of antigen, T cells lacking CD28 have reduced expression and activity of Aurora B (AIM1, Aurora-1), a serine/threonine kinase, described as a chromosome passenger involved in cytokinesis and chromosome architecture. It is thought that Aurora B kinases are responsible for chromatin modifications, including phosphorylating histone H3, however, there is no information on the role of Aurora B in T cells. To understand its function, we used retroviral transduction of Aurora B constructs. In vitro, dominant negative Aurora B expressed in activated wild type T cells decreased cell expansion at an early stage. Conversely wild type Aurora B transduced into responding CD28-deficient T cells enhanced early cell expansion during the phase of active division, but did not provide a long-term survival advantage. Interesting, wild type Aurora B co-introduced with Bcl-xL in responding CD28-deficient T cells enhanced cell expansion during the phase of active division, and provided a long-term survival advantage. These results indicate that Aurora B is induced by CD28 cosignaling and allows T cells to maintain cell division while synergizing with Bcl-xL to control T cell survival.

Vaccine Development & Immunology—II

CD8+ T Cell Subsets with Noncytotoxic Anti-HIV Activity

Presenter: Scott Killian, University of California, San Francisco

Collaborators: Sharon Ng, Jay A. Levy

Principal Investigator: Scott Killian

UARP Award Number: F05-SF-218

Background: The ability of CD8+ T cells to inhibit HIV replication through cytotoxic and non-cytotoxic mechanisms has been well described [1;2]. However, many features of the effector cell response remain unclear. Subset differences, regulatory effects, and priming efficiency could explain the observed variation in the quality of CD8+ T effector cell responses between individuals. We have previously investigated the non-cytotoxic effector properties of several CD8+ T cell subsets. Differential effector activity has been observed to be associated with CD28, HLA-DR, CD11b, and VCAM expression, but not CD38 and CD57 expression [3;4]. Importantly, we and others have observed that IL-2 is required for optimal inhibition of HIV replication by CD8+ cell non-cytotoxic effects [5-7]. These observations have heightened our interest in the investigation of IL-2 responsive CD8+ cells and potential CD8+ regulatory cell subsets.

Methods: CD8+ cells were isolated from the PBMC of HIV-1 infected subjects using immunomagnetic beads. These cells were then stained antibodies specific for various surface markers and sorted into phenotypically distinct populations using a FACSDiva. Antiviral activity was assessed upon coculturing the CD8+ cell subsets with acutely infected heterologous CD4+ cells. CD4+ cells, after 3 days of PHA stimulation, were infected with the CXCR4 tropic, chemokine resistant, primary HIV-1 isolate SF33. HIV replication was assessed by measuring the reverse transcriptase (RT) levels present in the cell culture supernatants at days 4 and 7.

Results / Expected Results: Our studies during the past month suggest that CD8+ cells with non-cytotoxic activity can be further distinguished by cell surface markers (e.g. CCR7). Our observations also indicate that other subsets of CD8+ cells may have the ability to negate or regulate this anti-HIV activity.

Conclusion: As these studies will better characterize the relationship between phenotype and CD8+ T cell immune responses, the proposed research can potentially be applied to improve future therapeutic and vaccine strategies.

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Vaccine Development & Immunology—II

Analysis of CD8low T cell Defects in HIV Infection

Presenter: Marc Schweneker, J. David Gladstone Institutes San Francisco

Collaborator: Joseph M. McCune

Principal Investigator: Marc Schweneker

UARP Award Number: F05-GI-219

Background: Infection with the human immunodeficiency virus type 1 (HIV) usually results in progressive disease that cannot be controlled by the immune system. Recent studies have demonstrated that infection is associated with the development of CD3+CD4-CD8+ T cells with low CD8 expression and impaired effector function. Preliminary results indicate that such "CD8low T cells" are defective in signaling across the T cell receptor (TCR) complex. Since these cells can comprise a large fraction of the CD8+ T cell compartment in both non-human primates infected with the simian immunodeficiency virus (SIV) and in humans infected with HIV, their presence may in large part explain the ineffectiveness of the anti-viral immune response.

Methods: To better understand functional deficits we will assess signaling pathways in human CD8low T cells. Conventional methods for the analyses of signaling pathways (e.g., Western blots) are limited in their ability to simultaneously measure multiple cellular parameters (e.g., cell lineage, phenotype, cellular activation, and protein phosphorylation status) within complex populations of cells of the immune system. Therefore, we implement and optimize a method to study multiple cellular parameters by flow cytometry, which will allow rapid and efficient analyses of signal transduction pathways in combination with phenotypical characterization of single cells within heterogeneous cell populations derived from primary sources. Using this method, TCR-mediated signal transduction will be analyzed in human CD8low T cells generated in the HIV-infected SCID-hu Thy/Liv mouse and in CD8low T cells from HIV-infected patients in varying stages of disease progression.

Results or Expected Results: Different conditions for the flow cytometric-based method were tested and optimized because different fixation and permeabilization protocols have been shown to affect measurements of phosphorylation events and cell surface staining. Optimized conditions will now allow us to analyze defects of TCR-mediated signal transduction in human CD8low T cells generated in the HIV-infected SCID-hu Thy/Liv mouse and to determine whether similar signaling defects are present in CD8low T cells from HIV-infected patients in varying stages of disease progression.

Conclusion: Results of the experiments will enable us to identify altered signal transduction events of CD8low T cells and specifically correlate these with cellular dysfunctions in the context of HIV infection. Information obtained may also suggest strategies by which such CD8+ T cell dysfunction might be prevented or reversed during the course of HIV disease progression. Thus, we will not only be able to assign a more precise molecular mechanism to the observed phenomenon of T cell dysfunction in HIV disease. We will hopefully be able to later use this information to suggest specific new therapies for HIV disease.

Vaccine Development & Immunology—II

Effector and Central Memory CD4 T-cell Responses during Anti-Retroviral Treatment Interruption

Presenter: Rachel Schrier, University of California, San Diego

Collaborators: Ronald Ellis, Scott Letendre, Deborah Durand and the HNRC

Principal Investigator: Rachel Schrier

UARP Award Number: ID03-SD-018

Background: Immune responses were studied for HIV infected individuals who chose to stop anti-retroviral treatment and agreed to frequent blood sampling. Study participants could request re-initiation of therapy at any time. One of the critical aspects of our study of CD4 T-cell immune responses to HIV and opportunistic infections during monitored treatment interruption was the study of immune response assays during changing antigen (HIV) levels. Comparison of T-cell cytokine expression (an effector memory (EM) response) versus proliferative capacity (a central memory (CM) response) is emerging as a critical distinction since it seems that at least for HIV and CMV, cytokine (Interferon gamma) expression reflects the presence of antigen and the ability to respond, whereas the capacity to expand (proliferate) when exposed to antigen correlates with protection from clinical disease by that pathogen

Methods: Proliferation and ELISpot assays were set up on the same day and the same antigens were used at identical concentrations. The same number of cells were plated in each assay (200,000. The number of cytokine expressing cells (ELISpot) was assayed for INFg and IL-2. Lymphoproliferation (LP) is mean of triplicate wells while ELISpot is mean of duplicate wells. Cut-offs for positive responses were >3 for LP and >5 for ELISpot.

Results: Our data (supported by this grant) on individuals who elect to stop anti retroviral therapy, has shown that the proliferative (CD4 T-cell) response to HIV remains strong in individuals who's virus has been suppressed by therapy for an extended period of time, and the proliferative response is not boosted by increasing HIV RNA levels when anti-retroviral therapy ceases. Also, the capacity to proliferate when exposed to HIV antigen in vitro while on therapy is inversely related to HIV RNA rebound levels off therapy (p=.04). Data gathered using ELISpot analysis (interferon gamma and IL-2) for HIV and opportunistic pathogens for the same samples is yielding very different results, confirming that capacity to proliferate and cytokine expression cannot be considered interchangeable assays. First, although it has been controversial as to whether IL-2 expression represents a CM or EM function, our findings are that the number of IL-2 expressing cells usually correlates with interferon gamma expressing cells (EM) for mitogen PHA responses. However, IL-2 expression is generally lower than interferon gamma for antigens, but does not necessarily correlate with ability to proliferate to the same antigen for the same donor. Second, some antigens which elicit a strong T-cell proliferative response in vitro (such as Candida) do not stimulate lymphocytes to express either interferon or IL-2, while Herpesvirsues such as HSV and CMV stimulate many cells to express interferon gamma, even in the absence of a proliferation response to the same antigen. As regards HIV reactivity in these HIV infected donors, numerous interferon gamma expressing cells is associated with a low CD4 T-cell proliferative response and a strong T-cell proliferative associated with low interferon gamma expression. Surface phenotype to characterize CM and EM CD4 cells, using CD45RA-CCR7+,- is also being analyzed.

Conclusions: In HIV infected individuals on anti-retroviral therapy, ability of CD4 T-cells to expand (proliferate) in response to HIV predicts comparatively lower viral set point if treatment is stopped. If T-cell proliferative response is compared to interferon gamma expression, a reverse relationship between the two assays is observed, suggesting a positive relationship between viral load and CD4 T-cells that express interferon gamma when exposed to HIV.

Vaccine Development & Immunology—II

Mechanism of Monocyte Neuroinvasion: Unique to HIV/SIV or Shared by Other Pathogens?

Presenter: Candice Clay, University of California, Davis

Collaborators: Denise S. Rodrigues, Manuela Raffatellu, Yan S. Ho, Somkanya Das, Ursula Esser

Principal Investigator: Candice Clay

UARP Award Number: D05-D-406

Monocytes are thought to transport human immunodeficiency virus (HIV) and its simian counterpart SIV across the blood-brain barrier (BBB) by a poorly defined mechanism, triggering events that can lead to neurological complications and development of neuro-AIDS. Other neuroinvasive pathogens, such as cytomegalovirus (CMV) and Listeria, similarly infect monocytes, however dissemination into the brain may occur by an alternate route. We hypothesize that SIV infection triggers a unique monocyte migratory program promoting enhanced neuroinvasion and CNS inflammation, distinct from monocyte trafficking characteristics initiated by infection with CMV or Listeria as well as Salmonella, a non-neuroinvasive pathogen. The specific objectives of the newly funded study are 1) to identify unique phenotypic and functional monocyte trafficking characteristics in SIV infection and 2) to delineate autocrine regulatory mechanism(s) in monocytes, such as production of proinflammatory chemokines, that are likely to contribute to continued monocyte brain recruitment.

To address these specific aims, we will conduct in vitro infection experiments and contrast migratory characteristics of monocytes infected with neurovirulent strains SIV/17E-Fr and SIV DeltaB670 to monocytes infected with rhesus CMV expressing enhanced green fluorescent protein (EGFP), Listeria monocytogenes wildtype strain 10403S or Salmonella typhimurium wildtype derivative IR715 (both transformed with a GFP-expressing construct). We will recapitulate the optimized conditions previously described for HIV infection to enhance susceptibility of rhesus monocytes to SIV infection in vitro. Freshly isolated monocytes will be cultured short-term in the presence of monocyte colony-stimulating factor and SIV-infected, prior to their differentiation and upregulation of macrophage marker CD71. For phenotypic and functional analysis, we established multi-color staining panels to define activation markers, chemokine receptor profiles and intracellular cytokine/chemokine production using flow cytometry. Assays of monocyte function in vitro will be complemented with characterization of infiltrating monocytes in brain tissues derived from acutely SIV-infected rhesus macaques (Clay et al, 2005).

Our anticipated results include expression of distinct chemokine receptors in rhesus monocytes infected with neurovirulent SIV strains, correlating with a neuroinvasive phenotype and with monocytes' suggested role as 'Trojan horse' for viral entry into the brain. This is supported by preliminary findings demonstrating increased CNS infiltration of fluorescein dye+ monocytes in acutely SIV-infected, but not uninfected macaques, following autologous cell transfer of fluorescein dye+ peripheral blood mononuclear cells after a 2-day in vivo migratory period. We also expect production of brain-specific chemokine signals in SIV-infected monocytes, such as fractalkine/CX3CL1 and MCP-1/CCL2, that are likely to promote continued monocyte brain recruitment in vivo, potentially shared by other brain-invading pathogens.

Determining unique monocyte migratory parameters triggered upon SIV infection (versus infection with other viral or bacterial pathogens) as proposed under this new award, will help delineate mechanism(s) of monocyte neuroinvasion. This may lead to the development of novel drug targeting options and treatment strategies that inhibit aberrant monocyte neuroinfiltration during HIV infection by targeting specific monocyte subsets and their trafficking signals.

Vaccine Development & Immunology—II

Exhaustion of Regulatory T Cell Subsets Induces B Cell Dysfunction in HIV Subjects

Presenter: Jennifer Snyder-Cappione, J. David Gladstone Institutes, San Francisco

Collaborators: Joan Chapman, Hugo Barbosa, Esper Kallas, Douglas Nixon

Principal Investigator: Jennifer Snyder-Cappione

UARP Award Number: F05-GI-209

Background: B cell hyperactivity, including hypergammaglobulinemia, is very common in HIV infected subjects. Interestingly, higher serum immunoglobulin titers are associated with rapid loss of CD4 T cells; this is believed to be due to increased formation of autoimmune complexes, which often contain the gp120 antigen and specifically target CD4 T cells for apoptosis. We hypothesize that regulatory T cells (Treg, NKT cells) directly suppress activation, proliferation, and immunoglobulin secretion by B cell populations. We propose that B cell aberrancies, including hypergammaglobulinemia and the excess of gp120-specific antibodies present in many persons infected with HIV, are a direct result of regulatory T cell dysfunction in these patients. It may be possible to abrogate the B cell dysfunctions present in many HIV-infected patients through enhancement of the suppressive mechanisms of regulatory T cells; this could lead to the generation of novel therapies that alter the frequencies and /or effector functions of regulatory T cells to reduce and/or prevent both CD4 T cell loss (induced by antibody complexes) and B cell diseases, including autoimmune disorders and lymphomas, that are present in afflicted individuals.

Methods: The frequencies of ASCs will be enumerated using IgG, IgM, and IgA Elispot assays. These assays can measure the total number of Ig secreting cells, as well as the frequencies of ASC secreting antibodies for specific viral or protein antigens. Total plasma titers for Ig isotypes will be measured by ELISA. The frequencies and functions of Treg and NKT populations will be evaluated by Flow Cytometry and Elispot assays.

Results/ Expected Results: Preliminary data suggest Treg cells are capable of directly inhibiting immunoglobulin secretion from ASCs in vitro. This novel 'B regulatory' effector function of Treg cells will be evaluated from PBMC of acute and chronic HIV-infected individuals to determine if there is a correlation between the ex vivo effector functions of regulatory T cell populations and the presence of B cell disorders, including lymphomas, found in many HIV-infected individuals. A subset of these infected patients will be studied longitudinally to determine if ex vivo Treg / NKT cell frequencies/functions are related to the rate of CD4 T cell loss and progression to AIDS.

Compendium Only

CXCR4-tropic Viruses Are Common among Antiretroviral-treated Patients with Detectable Viremia and Associated with Lower Treatment-mediated CD4 Gains

Principal Investigator: Steven Deeks, University of California, San Francisco

Authors: Peter Hunt, Jeffrey Martin, Michael Bates, Wei Huang, Serena Spudich, Richard Price, David Williamson, Elizabeth Sinclair, Rebecca Hoh, Steven Deeks

UARP Award Number: ID04-SF-012

Background: Among untreated HIV-infected individuals, CXCR4 (X4)-tropic viruses are uncommon except in advanced stages of immunodeficiency. However, little is known about the prevalence and immunologic consequences of X4 tropism in treated patients with detectable viremia.

Methods: The chemokine receptor tropism of plasma viral isolates was determined by the HIV Entry Assay (Monogram Biosciences) and compared between untreated and antiretroviral-treated chronically infected patients with detectable viremia sampled from two San Francisco cohorts. Differences between groups were adjusted for CCR5 delta32 genotype, nadir CD4+ T cell count, and duration of HIV infection. The association between tropism and naïve (CD45RA+CD62L+), activated (HLA-DR+CD38+), and non-activated memory CD4 counts was also assessed.

Results: Compared to the 81 untreated patients, the 186 treated patients had similar median CD4 counts (258 vs. 294 cells/mm3) and years since initial HIV diagnosis (13 vs. 12 years) but lower median plasma HIV RNA levels (3.6 vs. 4.0 log10 copies/ml) and nadir CD4 counts (60 vs. 203 cells/mm3). The treated patients had a median of 4 NRTI-associated and 2 major PI-associated mutations. Of 186 treated patients, 75 (40%) harbored dual/mixed (DM) or X4-tropic viruses compared to only 12 of 81 (15%) untreated participants, $p<0.001$. Among all participants, DM/X4 tropism was more common in those with lower current and nadir CD4 counts ($p<0.001$ for both comparisons) and in those heterozygous for the CCR5 delta32 polymorphism ($p=0.02$). Even after adjustment for nadir CD4 count, duration of HIV infection, and CCR5 delta32 genotype, treated patients had a 4-fold greater odds of DM/X4 tropism than untreated patients, $p=0.004$. Patients harboring DM/X4-tropic viruses had lower naïve ($p=0.05$) and resting memory CD4 counts ($p=0.02$) than those harboring R5-tropic viruses, but similar activated CD4 counts ($p=0.27$). Furthermore, after adjustment for plasma HIV RNA levels, treated patients harboring DM/X4 tropic viruses were maintaining 78 fewer CD4+ T cells/mm3 above their pre-treatment nadir than those harboring R5-tropic viruses ($p=0.002$).

Conclusions: Treated patients with partial viral suppression are more likely than untreated patients to harbor DM/X4-tropic viruses, independent of the extent of current or prior immunodeficiency. DM/X4 tropism is also associated with fewer treatment-mediated CD4+ T cell gains, perhaps due to a greater ability to deplete resting memory and naïve CD4 cells.

Compendium Only

HIV-Infected Outpatients: Potential Pneumocystis Reservoir

Principal Investigator: Laurence Huang, University of California, San Francisco

UARP Award Number: ID03-SF-027

Background: Pneumocystis pneumonia (PCP) remains a significant cause of morbidity and mortality in HIV-infected persons, particularly in populations with a lower prevalence of HAART and PCP prophylaxis use. At present, the natural reservoir of human Pneumocystis, P. jirovecii (formerly P. carinii) remains unknown. Knowledge of the reservoir might lead to novel prevention strategies. This study explores the hypothesis that HIV-infected patients may be a reservoir of P. jirovecii. Objectives: (1) To determine which of three polymerase chain reaction (PCR)-based assays offers the highest sensitivity for epidemiologic studies aimed at studying the natural reservoir of P. jirovecii; (2) To determine the proportion of patients without clinical PCP who have evidence of Pneumocystis colonization on oropharyngeal washing (OPW, gargling) specimens; (3) To identify potential risk factors for Pneumocystis colonization.

Methods: We performed a cross-sectional hospital-based study. Subjects were HIV-infected patients with suspected PCP who were undergoing sputum induction and/or bronchoscopy to diagnose PCP. Subjects completed a questionnaire designed to examine risk factors for Pneumocystis colonization and provided an OPW specimen by gargling 10 ml 0.9% NaCl for 60 seconds. Three different PCR assays were used to examine the OPW specimens. Two of the assays detect P. jirovecii DNA, while the third detects P. jirovecii mRNA. For this study, we focused on the subjects who were PCP-negative by microscopic examination of sputum or bronchoscopy specimens performed in the San Francisco General Hospital Microbiology lab.

Results: To date, we have enrolled 176 HIV-infected patients with suspected PCP who were undergoing diagnostic procedures for PCP; 57 subjects were PCP-negative. Among the three PCR assays, the assay targeting the P. jirovecii mitochondrial large subunit (mtlsu) rRNA appeared to have the highest sensitivity for detecting Pneumocystis in PCP-negative subjects. Overall, 34 (60%) of 57 PCP-negative subjects had evidence of Pneumocystis colonization at the mtlsu rRNA locus. Although no clinical factors predicted colonization with confidence, colonized subjects tended to have lower median CD4 cell counts (67.5 cells/μl vs. 105 cells/μl, p=0.54) and fewer were receiving PCP prophylaxis (28% vs. 43%, p=0.24).

Conclusion: The demonstration of Pneumocystis colonization in these PCP-negative patients suggests that humans may be a reservoir for P. jirovecii and the identification of risk factors for colonization could present new opportunities for disease prevention.

Compendium Only

Identification of Small Molecule Inhibitors of the Simian Immunodeficiency Virus NEF and the Cellular P21-activated Kinase

Principal Investigator: Earl Sawai, University of California, Davis

Authors: Scott Wong, Ruiwu Liu, Alan Lehman, Erwin Antonio, Michael Ye, Kit Lam, Earl Sawai

UARP Award Number: ID03-D-060

Nef, a viral protein encoded by the Simian Immunodeficiency Virus (SIV) and the Human Immunodeficiency Virus (HIV) has been shown to be important for pathogenesis in rhesus macaques and humans. Nef is capable of enhancing virion infectivity, down-regulating CD4 and MHC-I proteins from the cell surface and participating in cellular activation. We have shown that Nef binds and activates a member of the p21-activated kinase (PAK) family, a group of cellular serine-threonine kinases. Amino acid substitution mutants of SIV Nef that abrogate Nef-mediated PAK activation revert in vivo and these reversions correlate with a pathogenic outcome. These results demonstrate the importance of the Nef-PAK interaction to the virus.

To further study the Nef-PAK interaction, we have identified small molecule compounds that inhibit PAK activation by Nef. We have expressed and purified wild-type SIV Nef in E.coli as a poly-histidine tagged fusion protein and purified it by nickel affinity chromatography. Recombinant SIV Nef protein was used to screen one-bead one-compound combinatorial small molecule libraries to identify ligands that bind Nef. These libraries consist of functional small molecules attached to the outer surface of the bead while a modified peptide coding tag, identifiable by microsequencing, is attached to the bead interior. Beads with ligands that bind Nef were identified using a colorimetric method.

After screening ~400,000 compounds from four different libraries, we have identified several small molecules that bind to SIVmac239 Nef. Compounds were tested for their ability to inhibit Nef-mediated PAK activation using an in vitro kinase assay on SIV and HIV Nef from transfected COS-7 cells. One compound was found to inhibit a novel kinase activity localized to the SIV Nef N-terminus. Another family of ligands was found to inhibit SIV and HIV Nef activity by reducing Nef associated PAK auto-phosphorylation. These compounds can also inhibit the Nef-PAK interaction in an in vitro kinase assay performed on SIV-infected cells. Currently, enhancing compound specificity and potency are underway by synthesizing and screening focused small molecule libraries. Future experiments include determining if these compounds affect viral replication, infectivity and CD4/MHC-I down-regulation. If these compounds are effective in cultures, testing will continue in vivo with the rhesus macaque model for SIV pathogenesis. It is hoped that compounds identified in these studies will represent a new class of HIV inhibitors.

Compendium Only

K-bZIP of KSHV: Transcriptional Properties and Replication Functions

Principal Investigator: Yoshihiro Izumiya, University of California, Davis

Authors: Yoshihiro Izumiya, Hsing-Jien Kung

UARP Award Number: F03-D-206

Kaposi's sarcoma (KS) is the major malignancy occurring in HIV-associated AIDS patients. The etiologic factor for KS has been linked to a human herpesvirus, called Kaposi's sarcoma -associated herpesvirus (KSHV) or human herpesvirus type 8 (HHV8). KSHV genome is present in virtually all KS tumors. Serological studies of KS patients demonstrate that seropositivity precedes the onset of KS and correlates with increased KS risk. These results together suggest KSHV may play a key role in the development of KS. KSHV exists in two states in its host, a latent phase and a lytic cycle. In the latent state only restricted viral genes are transcribed, while in the lytic state more than eighty viral genes are transcribed in cascade fashion. The present study is aimed to understand the role of K-bZIP in the replication of KSHV. K-bZIP is a leucine-zipper protein encoded by KSHV. Previously, we found that one of the potential functions of K-bZIP is to modulate the transactivation potential of K-Rta, by repressing its function in a promoter dependent manner (J. Virol. 2003a). Further, we found K-bZIP directly interacts with CDK2-cyclin complex and inhibits its kinase activity. The interaction causes G1 cell cycle arrest and helps viral DNA replication take place, in the absence of competition by cellular DNA replication (J. Virol. 2003b). These results show that K-bZIP plays versatile roles in KSHV replication.

In this presentation, we focused on repression function of K-bZIP. Sumoylation has emerged as an important posttranslational modification that affects the location and function of cellular and viral proteins, and also plays a significant role in transcriptional repression along with Ubc9, the E2 sumo conjugation enzyme. We present evidence that K-bZIP is sumoylated at the lysine 158 residue and associates with Ubc9 both in a cell-free system and in virus infected BCBL-1 cells. The expression of K-bZIPK158R mutant, which was no longer sumoylated, exhibited the reduced transcriptional repression activity. This indicates that sumoylation plays an important part in the transcriptional repression activity of K-bZIP. Finally, we mapped K-bZIP binding sites on KSHV genome by chromatin immunoprecipitation experiments, and demonstrated that K-bZIP interacts with and recruits Ubc9 to specific KSHV promoters. Thus, our data indicate that K-bZIP is a Sumo-adaptor, which recruits Ubc9 to specific viral target promoters thereby exerting its transcriptional repression activity. The information provides a framework to understand the complex role of K-bZIP and its posttranslational modification by sumoylation in KSHV replication.

Health Services, Policy & HIV/AIDS Treatment and Care 2

HIV/AIDS Policy and Health Care Financing Research

Going Global: The Challenges of Translating U.S. HIV Research to a Resource-limited Setting

Presenter: Joanna Crane, University of California, San Francisco

Principal Investigator: Joanna Crane

UARP Award Number: D04-SF-401

Effective antiretroviral therapy has been available to patients in the U.S. for nearly 10 years and has resulted in remarkable improvements in the longevity and quality of life of people living with HIV. As the virus has become manageable among their own patients, many U.S.-based AIDS researchers and clinicians have begun extending their research interests beyond U.S. borders in an attempt to address the global epidemic. As a result, a growing number of American AIDS researchers are now involved in projects in sub-Saharan Africa, which bears the burden of over 70% of the world's HIV infections.

As these researchers confront the African epidemic, they encounter a landscape of disease that is both eerily reminiscent of places like San Francisco in the 1980s and at the same time is radically different from the epidemic as they know it. This paper is a preliminary exploration of some of the challenges involved in such research, using qualitative data collected through interviewing and observing AIDS researchers in California and Uganda. The data presented here is a small part of a larger dissertation project focused on the science and global politics of HIV treatment and drug resistance.

This paper will present examples of what I'm calling the problem of "translatability." "Translatability" refers to the ways in which knowledge about HIV/AIDS gleaned from the American experience does or does not translate to a Ugandan context. Specifically, I will discuss problems of "biological translation" that arise in relation to measuring biological markers of HIV disease across contexts and problems of "ethical translation" that arise in attempting to apply Western standards of care to a resource-limited setting. Understanding these problems of translation may assist researchers in both the U.S. and developing countries to negotiate the challenges of collaboration and improve their ability to conduct relevant and accurate HIV research.

HIV/AIDS Policy and Health Care Financing Research

Pilot to Transition Incarcerated HIV+/– Opiate Users to the Community

Presenter: Paula Lum, University of California, San Francisco

Collaborators: Mary White, Ross Jamison

Principal Investigator: Jacqueline Peterson-Tulsky

UARP Award Number: ID05-SF-040

The decrease of risky sexual and injection drug use behaviors have been the primary focus of prevention efforts against the spread of the HIV. For opioid addicts, abundant data has shown the reduction in risky behavior when treatment is offered with opioid replacement therapy. For more than three decades Methadone, administered in a structured strictly prescribed program, was the only treatment available until the release of Buprenorphine. California laws have restricted opioid replacement therapy options in many communities, and this has made it virtually impossible for jails to begin opioid replacement therapy on incarcerated opioid addicts. Physicians can now prescribe effective opioid replacement therapy for opioid dependent persons using Buprenorphine, including persons who are incarcerated. In San Francisco, leadership in public health and in the sheriff's department has been supportive of providing opioid replacement therapy to HIV+ and HIV- addicts, but the program in the jail has been slow to be implemented.

Buprenorphine is viewed as an effective, economical and viable alternative to methadone, especially in a jail setting where the complex rules of methadone licensing may be difficult to implement. But, Buprenorphine has yet to be fully utilized in a jail-based setting as opiate replacement. Initially, the San Francisco Jail Health Services program for treating opiate dependent incarcerated persons was administered to opiate dependent individuals undergoing opioid withdrawal in jail. Upon release, each individual was referred to the San Francisco Department of Public Health's Induction Clinic (OBIC), located in the Mission District of San Francisco. However, a simple referral to OBIC has not been effective for linking released inmates to ongoing Buprenorphine care.

Medication adherence and treatment outcomes are optimized when linked with substance abuse treatment. The largest challenge is to stabilize peoples' lives so that they can consistently access care and to provide comprehensive, quality care to those whose lives remain chaotic. As Ryan White-funded primary care providers, the PHP clinic provides substance abuse counseling and has access to substance abuse treatment for its patients through multiple community linkages and collaborations. Substance abuse services are coordinated by five full-time social workers. Services include on-site substance abuse counseling, referrals to inpatient and outpatient substance abuse treatment programs, and a strong linkage to OTOP on the hospital campus.

Concurrent studies of this population in San Francisco indicate that a lack of ongoing effective opiate replacement therapy carries a direct correlation with high rates of progression from HIV to AIDS diagnosis and death, recidivism, risky behavior and subsequent spread of infectious diseases such as HIV and HCV. This study is designed to evaluate linkages between opiate replacement therapy in jails and community healthcare. Our research goals for this pilot are: (1.) to document the implementation of a program to induce and maintain HIV positive and negative inmates on Buprenorphine while in jail, (2.) to implement a transition program for jail inmates on Buprenorphine into the community, and (3.) to describe the characteristics of HIV positive persons, including changes in reported HIV risk behavior, who continue on Buprenorphine after release from jail.

HIV/AIDS Policy and Health Care Financing Research

The Effect of State Cost Containment Strategies on the Insurance Status and Health Care Use of HIV Infected People

Presenter: Neeraj Sood, RAND Corporation, Los Angeles

Principal Investigator: Arleen Leibowitz, University of California, Los Angeles

UARP Award Number: HP04-LA-902

Background: In order to balance their budgets, many states are considering reducing eligibility for Medicaid, one of the fastest growing state expenditure categories. Using variation in state policies, this paper models the effect of more stringent eligibility criteria for Medicaid on the insurance status and the use of antiretroviral therapy (HAART) for people living with HIV (PLWH). The paper also investigates whether there are differential effects of the state policies for those who are disabled among the PLWH.

Data: The data is from the HIV Cost and Services Utilization Study (HCSUS), a nationally representative probability sample of adults receiving care for HIV/AIDS in 1996 in the contiguous United States. The sample used in this paper includes all 2,864 respondents, sampled with known probability at each stage. For this analysis, the HCSUS data was merged with a separate database on states' Medicaid eligibility rules and other state-level covariates like unemployment and uninsurance rates.

Methods: Multinomial logistic regressions were used to derive the predicted probabilities of various types of insurance and the marginal effects of relaxing states' Medicaid eligibility rules on the insurance status of PLWH. For the second analysis involving HAART use, reduced form logit regressions were estimated. Both analyses were carried out for the full sample, as well as for various subsets of the sample, using disability and work status to differentiate among patients.

Results: The results suggest that restricting eligibility increases uninsurance and has no effect on private coverage for PLWH. Further, more stringent eligibility criteria have a larger effect on the uninsurance rates of the disabled and an even larger effect for those who are both disabled and unemployed. The same holds true for the analysis involving HAART use.

Conclusion: Lower eligibility thresholds are likely to raise uninsurance rates and reduce the use of antiretroviral therapy among PLWH - more so, for those who are disabled and unemployed. There is no evidence of a "crowding out" effect of public insurance on private coverage for PLWH.

HIV/AIDS Policy and Health Care Financing Research

The Impact of Medi-Cal Restructuring on Care of Beneficiaries with HIV/AIDS

Presenter: David Zingmond, University of California, Los Angeles

Collaborators: William Cunningham, Thomas Rice, Richard Hector

Principal Investigator: William Cunningham

UARP Award Number: HP04-LA-903

Background: Medicaid is the principal payer of care for Americans living with advanced HIV-infection. Individual state Medicaid programs have promoted managed care plans (MCPs) over traditional Fee-For-Service (FFS) for controlling healthcare costs for individuals with chronic disease, such as persons living with HIV. California has the second largest cohort of HIV-infected Medicaid enrollees in the country and has introduced MCPs on a county-by-county basis. We examine the impact of MCP enrollment on mortality, hospitalization, changes in enrollment and cost among Medicaid beneficiaries with AIDS.

Methods: This study uses a retrospective longitudinal cohort study of 12,078 Medicaid beneficiaries with AIDS in urban counties of California, enrolled in January 1, 1999 and followed through December 31, 2003. We are analyzing Medi-Cal enrollment data linked to Medi-Cal claims, state hospital discharge abstracts, the state death certificate registry and the state AIDS Registry. The impact of MCP enrollment on mortality, hospitalization and changes in enrollment is estimated using multivariate regression models.

Results: In preliminary results, we identify 12,078 individuals, with 14.5% enrolled in MCPs. Characteristics of enrollees were: 83% male, age 41.5 years old (mean), 4.7 years since AIDS diagnosis (mean), 46% non-Latino White, 28% non-Latino Black, and 24% Latino. One third of the enrollees were hospitalized in 1998. During the study period, 63.3% of enrollees were hospitalized and 23.3% died. Rates were similar among MCP and FFS enrollees. However, 24.3% of MCP enrollees changed to FFS by the end of the period, while only 5.6% of FFS enrollees changed to a MCP. Multivariate regression models found no significant relationship between MCP enrollment and mortality, but MCP enrollment did appear to be associated with lower rates of hospitalization. MCP enrollment was associated with a greater odds of changing plans (Odds Ratio: 6.81, 95% Confidence Interval: 5.71 to 8.13).

Conclusions: Similar death rates with lower utilization among managed care recipients suggest that MCP enrollment may reduce hospitalization rates. Unmeasured severity may bias these estimates, suggesting benefit where there is none. Greater likelihood of disenrollment from MCPs is suggestive of low patient satisfaction or difficulties with access to care. Before wholesale changes are made in the delivery of care, policy makers should address whether non-clinical measures such as cost, satisfaction and access to care differ substantially between MCP versus FFS Medicaid for HIV-infected and other chronically ill beneficiaries. Future work will focus on revising estimates of the impact of MCP on outcome, comparing the cost of care for current MCP and FFS enrollees and examining physician readiness where mandatory MCP enrollment would be instituted.

HIV/AIDS Policy and Health Care Financing Research

Insurance Purchase and Continuation Programs/ CARE-HIPP

Presenter: Arleen Leibowitz, University of California, Los Angeles

Principal Investigator: Arleen Leibowitz

UARP Award Number: HP04-LA-901

Background: CARE-HIPP (Health Insurance Purchase Program) is a California State program that purchases private, continuation health insurance for persons living with HIV (PLH) in lieu of directly providing them antiretroviral medication through the AIDS Drug Assistance Program (ADAP).

Methods: This project examines both the cost-effectiveness of the current program and the potential to expand it. This research involves a number of steps: (1.) estimating the numbers and characteristics of current CARE-HIPP recipients; (2.) calculating the full cost of providing coverage for antiretroviral medication to CARE-HIPP enrollees through the premium purchase program and through ADAP; (3.) determining what the cost of providing antiretroviral medication to this population through ADAP coverage uniquely would be in the absence of private insurance purchase; (4.) calculating the cost-effectiveness ratio by comparing incremental costs and benefits; and (5.) making comparable calculations for populations of PLH who may reasonably be expected to have the option of continuing their private health insurance policies, namely those PLH with private insurance other than CARE-HIPP who currently also receive some ADAP assistance.

Results: The current CARE-HIPP program is highly cost-effective. It not only is less costly to provide antiretroviral medication through CARE-HIPP than directly through ADAP, but CARE-HIPP also provides coverage for physician and hospital services. The program would also be cost-effective for likely expansion populations.

Conclusions: The CARE-HIPP program should be continued and expanded.

HIV/AIDS Policy and Health Care Financing Research

Analysis and Mapping of Pharmacy Access to Syringes (AMPAS)

Presenter: Valerie Rose, Public Health Foundation Enterprises, Inc.

Collaborator: Glenn Backes

Principal Investigator: Valerie Rose

UARP Award Number: CR05-PHFE-801

Background: Landmark legislation (SB 1159), that permits California pharmacies to sell up to ten syringes without a prescription to anyone over the age of eighteen, was signed into law by the Governor in January 2005. The legislation also allows individuals to legally possess syringes. Although SB 1159 is a state-wide initiative, the legislation requires any interested local health department to opt in by establishing a "Disease Prevention Demonstration Project" for the sale and safe disposal of needles and syringes. To date, 13 of the 61 health jurisdictions in California have established pharmacy access programs, resulting in >100 participating pharmacies throughout the State. Twenty counties are in the planning stages, and 18 have refused to adopt local programs.

Methods: This is a community collaborative project between Public Health Foundation Enterprises and the Drug Policy Alliance. The San Francisco Department of Public Health AIDS Office is an additional collaborator. Using a case study design, we will explore the factors that facilitate or inhibit local implementation of SB 1159. Naturalistic inquiry will guide qualitative data collection through in-depth interviews with county health department, needle exchange program staff and policy makers. Purposeful sampling will be used to select 10 to 15 counties with low, medium and high prevalence of HIV/AIDS among IDU. Additional criteria for selection will include counties with sanctioned, unsanctioned or no needle exchange programs, early adopters into the pharmacy access program and non-adopters of pharmacy access. In the second year of the study, we will use existing quantitative data from three to five counties to conduct geographic analysis to map the distribution of HIV/AIDS among IDU in relation to syringe access. We will map the intersection of service needs and use and create overlays of service locations, such as participating pharmacies and needle exchange sites with patterns of HIV/AIDS density by risk-group to determine if services sites are in the proximity of populations most in need. Sampling will prioritize counties that can produce appropriate data variables (e.g., census tract level HIV/AIDS surveillance and/or substance use treatment data). In these counties, depending on the presence of needle exchange programs and the feasibility of collecting program data from needle exchange sites, information regarding pharmacy utilization for syringe purchases will enhance the richness of the GIS mapping component. The specific aims of the study are to: (1.) enumerate policies, procedures and systems for local implementation of SB 1159 and describe the context of implementation, (2.) describe county characteristics regarding needle exchange policies and syringe disposal practices, (3.) describe facilitators, barriers and dimensions of implementation or refusal to adopt local legislation, (4.) determine the proximity of participating pharmacies in relation to high incidence IDU neighborhoods, and (5.) develop and disseminate prototypes of successful strategies and policy toolkits.

Results: Cases studies will be summarized and formatted as toolkits to illustrate best practice approaches for implementation and evaluation. Data from the case studies will provide guidance to other California counties that are currently in the planning stages for local implementation of pharmacy access to syringes. The results will provide useful information and policy strategies for counties that have experienced political opposition to local implementation. Dissemination strategies include posting results on multiple websites and providing written monographs to interested counties throughout California.

Psychosocial and Mental Health Issues and HIV/AIDS Care and Treatment

The Relationship of Stigma to Psychological and Physical Well-Being in People with HIV/AIDS

Presenter: Gregory Herek, University of California, Davis

Principal Investigator: Gregory Herek

UARP Award Number: ID04-D-025

Background: This study examined how people with HIV/AIDS (PWHIVs) experience HIV-related stigma; how stigma experiences are associated with indicators of social, psychological, and physical well-being; and, how other variables affect that association. Stigma is conceptualized as having three key components for PWHIVs: (1.) experiences with enacted stigma (being the direct target of interpersonal rejection, discrimination, and other types of prejudicial behavior based on one's HIV status), (2.) felt stigma (beliefs about the nature and extent of enacted stigma), and (3) internalized stigma (shame, self-blame, and other negative feelings toward the self as a result of having HIV). Previous research on the social psychology of stigma suggests that felt stigma is an especially important construct because expectations of discrimination and ostracism often lead people with a stigmatized condition to avoid enactments of stigma by altering their behavior, often in ways that have deleterious consequences.

Methods: Self-administered questionnaires were completed by 200 PWHIVs recruited through the East Bay AIDS Research Institute (EBARI) at the East Bay AIDS Center (Berkeley) and the Alameda County Medical Center (Oakland). The sample included equal numbers of men and women (with two transgender respondents); was 72% African American, 18% White, and 7% Hispanic; was 39% gay/bisexual and 57% heterosexual; and had a mean age of 45 years and a median income of $5,000 - $15,000.

Results: Based on factor- and item-analyses of questionnaire responses, brief measures of enacted stigma, felt stigma, and internalized stigma were constructed (coefficient alpha > .80 for all scales). Correlational analyses revealed that all three forms of stigma were significantly associated with psychological distress, impaired health status and other variables related to well-being. Multivariate analyses indicated that the association between stigma and psychological/physical distress was due mainly to the strong connection between felt stigma and the outcome variables. Preliminary analyses suggest that this linkage is partially mediated by the coping strategies adopted by individuals with high levels of felt stigma. These strategies involve avoidance of others and active hiding of one's HIV status. Analyses of the role of other relevant intervening variables will also be presented.

Conclusion: The experience of stigma is significantly associated with physical and psychological well-being in people with HIV/AIDS. Clinical and institutional implications of the results will be discussed.

Psychosocial and Mental Health Issues and HIV/AIDS Care and Treatment

Effects of Unknown Infant HIV Serostatus on Maternal Perceptions of Stress, Distress, Social Support, and Maternal and Baby Adherence to HIV Medications

Presenter: Maureen Shannon, University of California, San Francisco

Principal Investigator: Maureen Shannon

UARP Award Number: D05-SF-410

Background: The primary objective of the study is to describe perceptions of maternal uncertainty, stress and distress in HIV-infected women in association with the unknown HIV status of their infants and to document changes in these variables over time. Secondary objectives include determining relationships between maternal social support and perceptions of stress and distress; and between levels of maternal stress and distress and adherence to antiretroviral medications in the mothers and their infants. In California, the majority of women with HIV infection are in their childbearing years. HIV-infected women who become pregnant receive highly active antiretroviral therapy (HAART) both to reduce perinatal HIV transmission as well as to delay maternal disease progression. The use of HAART significantly reduces the risk of perinatal transmission from 25% to less than 2%. As a result, the vast majority of HIV-infected pregnant women are expected to give birth to uninfected, healthy infants. However, the HIV status of these infants is not immediately known and requires several HIV-specific viral laboratory tests during the first few months of life to determine their infection status. Ninety-nine percent of infected infants are diagnosed during the first four months of life and negative HIV viral test results during this time can be reassuring to parents. However, maternal perceptions of uncertainty about an infant's HIV infection status during the testing period can contribute to psychological and physiological distress. Perceptions of stress and distress in HIV-infected individuals have been associated with erratic adherence to antiretroviral medications, alterations in immune function and increased mortality rates. In addition, inconsistent maternal adherence to HIV medications has been linked to sub-optimal administration of HIV prophylactic medications to perinatally-exposed infants, thereby increasing the risk of infection in these infants.

Methods: This is a prospective, repeated measures study of HIV-infected mothers and their infants designed to describe the effects of unknown infant HIV status on: (1.) maternal perceptions of uncertainty; (2.) maternal perceptions of stress; (3.) maternal perceptions of psychological distress; (4.) maternal social support; and (5.) adherence to maternal and infant HIV medications. Study variables are measured in women at six time points (once during the third trimester of pregnancy and five times after delivery). Data are collected using standardized questionnaires and open-ended questions. Maternal and infant medical records are abstracted to confirm their health status. Data analyses include repeated measures analysis of variance for quantitative responses and content analysis for qualitative responses.

Results/Expected Results: This is a recently UARP-funded study with limited data analyses due to the small sample size. Demographic data for the 12 enrolled maternal subjects are as follows: mean age = 31.9 (range = 21-43); ethnicity/race = 41% Black (non-Hispanic), 41% White (non-Hispanic), 9% Hispanic, 9% Native American/Hispanic; HIV/AIDS stage: 33% with an AIDS diagnosis prior to the current pregnancy. Five maternal subjects have completed data collection, and all of their infants are uninfected by HIV DNA PCR testing.

Conclusions: This study has been designed to investigate aspects of maternal stress, distress, coping and medication adherence that have not been addressed previously in HIV-infected childbearing women. Information gained from this study will provide a foundation for developing interventions that may improve maternal and infant health outcomes during this potentially stressful period.

Psychosocial and Mental Health Issues and HIV/AIDS Care and Treatment

Acceptance Therapy to Reduce Avoidance Coping in HIV/AIDS

Presenter: Elizabeth Gifford, San Mateo Medical Center

Principal Investigator: Elizabeth Gifford

UARP Award Number: ID05-SMCHC-046

Background: Mental health problems are prevalent in patients with HIV/AIDS. A recent survey of 210 HIV infected patients treated in the San Mateo Medical Center found that 56% met diagnostic criteria for at least one psychiatric disorder (e.g., post-traumatic stress disorder, depression, or acute stress disorder). Of these patients, 37% met criteria for two psychiatric disorders and 31% met criteria for of all three disorders. Although every effort is made to provide psychiatric treatment to these patients, the above study found that the majority of patients (59%) received no mental health treatment. Unfortunately, even when patients do receive mental health treatment, these treatments have not been designed for patients suffering from multiple issues with complex health needs. Most empirically supported mental health treatments have been developed and tested in carefully controlled populations with only one mental health disorder and are not aimed at patients facing severe medical and health challenges with significant psychiatric comorbidities. The health costs of ongoing high-risk behavior are particularly high for HIV/AIDS patients, and their coping resources are burdened by heavy demands. Therefore, HIV/AIDS patients with psychiatric problems need mental health treatment that can help them improve their functioning across multiple life domains and disorders at the same time. Avoidance coping has been identified as an important mediator of emotional distress, comorbid psychiatric conditions, reduced quality of life, reduced physical health, and poor medication adherence in patients with HIV/AIDS. Such maladaptive avoidance is a common problem across anxiety disorders, depressive disorders, substance use disorders, chronic pain, as well as normal emotional responding to HIV infection (e.g., guilt, anger, sadness, internalized stigma) and issues with medical care (e.g., problems with medication adherence). Fortunately, behavioral acceptance-based treatments that attempt to decrease avoidant responding, such as Acceptance and Commitment Therapy and Dialectical Behavior Therapy, have been shown to improve a variety of disorders including depression/anxiety disorders, substance use, and chronic pain, among others. Acceptance-based behavioral treatments attempt to reduce patients' unhealthy avoidance behaviors (e.g., using substances or alcohol, engaging in risky sexual behavior, isolating socially, restricting physical activity, failing to comply with medical care and medication regimens, etc.) and to increase their ability to engage in adaptive behaviors, such as health related self-care, even when such actions feel difficult and distressing.

Methods: We will develop an acceptance-based behavioral treatment to empower HIV-infected patients to reduce avoidance coping and make constructive behavioral choices consistent with their health needs. We will pilot test this intervention as an addition to treatment as usual in a public primary care clinic for patients with HIV/AIDS. This therapy is innovative in its attempt to treat a patient population with mixed mental health problems with a single therapy targeting a core problem that decreases health across psychiatric domains.

Results: We anticipate that treatment targeting avoidance coping will both reduce avoidance coping and improve quality of life for patients with HIV/AIDS and mental health problems.

Conclusion: Adapting pre-existing acceptance based behavior therapies to the needs of HIV/AIDS patients with various mental health disorders may provide an effective treatment for the complex and mixed groups of multiply diagnosed patients seen in the majority of health care clinics.

Psychosocial and Mental Health Issues and HIV/AIDS Care and Treatment

Measuring and Understanding HIV Treatment Expectancies

Presenter: Mallory Johnson, University of California, San Francisco

Principal Investigator: Mallory Johnson

UARP Award Number: ID04-SF-082

Background: In a wide range of illness contexts, expectancies about treatment outcome play an important role in patients' health behaviors such as accessing care and adhering to treatments. In the context of HIV treatment, we know from cross-sectional findings that expectancies about benefits of treatment and confidence in one's ability to adhere to antiretroviral medications (ARV) are associated with adherence, which is related to clinical outcome. There is also strong concern that expectancies about reduced transmissibility resulting from antiretroviral treatment may lead to increased sexual risk among HIV+ persons on ARV. There is currently no psychometrically sound measure of HIV treatment expectancies that can be used to ascertain the impact of expectancies on adherence and transmission risk behavior. Measures used in prior research were either focused on disease progression (without regard for treatment expectancies) or were not developed using standard scale development protocols.

Methods: Guided by a modified Theory of Planned Behavior and utilizing a combination of qualitative and quantitative methodologies, we are developing an innovative measure of HIV treatment expectancies. We are following rigorous scale construction procedures and rely on previous research and measurement of HIV expectancies and the proposed qualitative research in the development of potential content and comprehensive statistical analytic expertise in the finalization of the measure.

Expected Results: We are currently conducting qualitative interviews with the target population and preliminary data analysis is underway.

Conclusion: This measure will target multiple dimensions of expectancies, including treatment efficacy, adherence self-efficacy, side effects and reductions in transmissibility resulting from treatment. This measure can then be used in larger studies to reliably explore the role expectancies in adherence, clinical outcome and treatment optimism (beliefs that treatment advances have negated the need to practice safe sex). Therefore, the research needed to develop this measure is a critical step towards investigating these important issues in larger observational and intervention studies.

Psychosocial and Mental Health Issues and HIV/AIDS Care and Treatment

Understanding the Role of Social Support Networks on Engagement in HIV Care among Publicly Insured Latinos and African Americans in Los Angeles County

Presenter: Amy Wohl, Los Angeles County Department of Health Services

Principal Investigator: Eric Bing, Charles R. Drew University of Medicine and Science

UARP Award Number: CH05-DREW-616

Background: Few studies have examined the role of social support networks on engagement in HIV treatment among low-income Latinos and African Americans. The successful management of HIV disease requires regular medication use and appointments with medical care providers, and these necessities are likely to impose substantial lifestyle adjustments on HIV-positive people, their families and their friends. For this reason, it is imperative to gain a better understanding of the nature and impact of social support networks on engagement in HIV treatment. The specific aims of the study are: (1.) to describe the engagement patterns in HIV treatment among publicly-insured Latinos and African Americans with HIV infection in Los Angeles County (LAC); (2.) to describe and evaluate the potential roles of formal and informal social networks in promoting engagement in HIV treatment; and (3.) to test for ethnic differences between Latinos and African Americans in the previous two aims. Our main hypothesis is that patients with stronger ties to family, friends and church networks are more likely to be engaged in their HIV medical treatment.

Methods: This study uses qualitative and quantitative methods to assess the impact of social support on engagement in HIV care among Latinos and African-Americans in LAC. The qualitative phase will consist of in-depth interviews that focus on domains central to engagement in HIV care. These domains include: social support from friends and family, social networks, social support and stress from family and friends, religious support and stress, and other HIV services such as case management or peer support groups. The qualitative interviews will inform the development of the survey instrument to be used in the quantitative phase. The quantitative phase will utilize a cross-sectional design to collect survey data from approximately 700 HIV patients. We are presently in the qualitative phase of this study. A total of 24 qualitative interviews will be conducted: 12 at the Rand Schrader HIV Clinic and 12 at the King/Drew Medical Center. The sample of HIV-positive adult participants will consist of six Latina women, six African-American women, six Latino men who have sex with men (MSM), and six African American MSM. Each interview will be audio-taped and last approximately 90 minutes. Interviews will be conducted in both Spanish and English. To analyze the data from the qualitative interviews, the interviews will be transcribed into English from the audio-tapes and entered into an Atlas.ti program. Codes will be identified and tagged to all excerpts of text. The coded text will then be grouped into thematic categories. The various thematic themes identified will be considered as potential content areas for inclusion in the quantitative survey.

Conclusion: This study will help better characterize the role of social support networks in promoting the engagement in care by Latinos and African-Americans living with HIV/AIDS. A better understanding of formal and informal social networks influence engagement in care may also provide insight into unique intervention strategies to improve engagement in care among HIV-positive Latinos and African-Americans.

Psychosocial and Mental Health Issues and HIV/AIDS Care and Treatment

Integrated Pain Management for Patients with HIV Receiving Care in a Public Setting: Preliminary Results

Presenter: Jodie Trafton, Palo Alto Institute for Research & Education

Collaborators: Dennis Israelski, John Sorrell, Percy Link

Principal Investigators: Jodie Trafton, Dennis Israelski

UARP Award Number: CR04-PAIRE-519

Background: Under treatment of pain is a serious problem in HIV positive patients, with 60-75% of patients reporting problems with pain. Pain is responsible for increased distress, disability, and health care use and decreased quality of life in persons living with HIV/AIDS. Alternative strategies to manage pain are urgently needed to improve healthcare outcomes in patients with HIV/AIDS. Recently, a program of cognitive-behavioral/relaxation-based group therapy was demonstrated to reduce patients' pain and distress. However, when this treatment was provided outside patients' normal place of care more than half of all patients did not complete the treatment program. We proposed to incorporate this promising treatment into patients' current HIV treatment programs, thus increasing patients' access to care and HIV treatment clinicians' exposure to a pain specialist.

Methods: An evaluation of the acceptability and effectiveness of integrating a cognitive-behavioral group therapy program for chronic pain into HIV/AIDS primary care. A pre-post analysis of pain and mental health outcomes is being conducted for 100 patients enrolled into the therapy groups at three HIV/AIDS primary care clinics. Medical record review is being conducted every three months to provide feedback to clinicians on improvements and shortcomings in pain assessment and treatment provided at the clinics.

Results: A therapy manual was developed and this 12-session treatment was offered at three primary care HIV/AIDS clinics, two clinics run by San Mateo County and a third clinic at the VA Palo Alto. Medical record review suggested that clinical providers assessed greater than 90% of patients for chronic pain problems with no biases in rates of assessment across populations. On-going feedback to providers about their referral patterns, inclusion of the clinic psychologist in team meeting, and support from the clinic directors has been associated with increasing rates of referral of patients with pain to the therapy. Here we report preliminary findings from the patients who have completed the first 12 weeks of enrollment. Fifteen of 18 patients completed all research assessments. A diverse population of patients enrolled (53% male, 47% female; 47% black, 33% white, 7% Hispanic, 13% other). Sixty percent of enrolled patients had a history of illicit drug use, including 27% with a history of illicit opioid use and 33% with a history of illicit use of prescription medications. Patients attended an average of 6.4 +/- 4.2 of a possible 12 group therapy sessions. Significant reductions in total score, pain intensity, fear and negative affect and increases in mobility were observed on the Pain Outcomes Questionnaire. Average pain intensity decreased from 7.5 +/- 1.9 at treatment start to 4.3 +/- 3.0 after 12 weeks. Depression scores were reduced to 40% of initial levels as measured by the Brief Symptom Inventory.

Conclusions: This therapy is feasible and well-accepted by both patients and clinicians when integrated into a primary care setting. Preliminary analysis suggests that enrollment in this therapy is associated with reductions in pain, and negative affect and increases in mobility in patients with HIV/AIDS.

Health Services Research and Quality of HIV/AIDS Care

Development of a Cultural Competency Training to Improve Clinician HIV/AIDS Screening and Education for Limited English Proficiency Latinos

Presenter: Stergios Roussos, San Diego State University

Collaborators: Salvador Sandoval, Felicia Batts, Yescenia Espinosa, Melbourne Hovell, Carol Sipan

Principal Investigator: Stergios Roussos

UARP Award Number: CR04-SDSUF-517

Background: Disparities in HIV/AIDS preventive and clinical care due to linguistic and cultural barriers are known and exist among California's large number of Latinos with limited English proficiency (LEP). Clinicians who serve LEP patients may not be prepared to discuss sensitive issues necessary to screen for behavioral risk factors among patients who are asymptomatic for HIV/AIDS. A prevention study is proposed to examine if health centers whose staff receive cultural competency training focused on HIV/AIDS for LEP Latinos increase their overall rate of screening of HIV behavioral risk factors and HIV testing among asymptomatic or unknown HIV status, LEP Latinos. The presentation will describe the development and pilot testing of the training to help improve screening and education of LEP Latinos for HIV/AIDS.

Methods: The development of the training was built on: (1.) review of similar trainings with published results, (2.) identification of recommended clinical guidelines and evidence-based practices for clinician-led HIV/AID prevention, (3.) cultural competency and diversity training, (4.) literature on sexual beliefs and practices among Latin Americans, with a focus on Mexicans, (5.) pilot data from clinician HIV/AIDS clinical practices, and (6.) consultation with HIV/AIDS clinician training experts in the USA and Mexico. Content and procedures of the training were presented and critiqued by research and clinical staff prior to formal implementation.

Results: Most clinician training materials and content identified for the development of this study's training was focused on providers who care for patients with HIV/AIDS (rather than prevention). Limited items were found to explicitly address health disparities and cultural and linguistic factors that may be address to facilitate clinician HIV/AIDS screening and education among LEP Latinos. Review of similar cultural competency trainings (found for non-HIV/AIDS issues) and conversations with research and clinical experts led to the development of role plays and interactive exercises intended to increase practice and retention of skills among clinicians and to increase comfort in discussing sexual health with LEP Latinos, with and without an interpreter. Trainings will include a formal four-hour workshop, booster sessions and case study presentations at monthly meetings.

Discussion: Given California's large and growing Latino population it is critical to address cultural and linguistic barriers to clinician screening and education of HIV/AIDS among Latino patients. This presentation will examine the development of a training to improve such screening and education. Sexual health screening and education can be adapted to better attend to customs, beliefs and values in Latin American culture. Ethical issues related to sexual health screening will be discussed, especially when using bilingual staff and family for interpreting. The presentation will outline challenges and opportunities for intervention at the provider and organizational levels that may lead for more culturally and linguistically appropriate care for of HIV/AIDS among diverse populations.

Health Services Research and Quality of HIV/AIDS Care

Screening for Acute HIV Infection in High Risk Men in Los Angeles County

Presenter: Peter Kerndt, Los Angeles County Department of Health Services

Collaborators: Lisa V. Smith, Apurva Uniyal, Robert Bolan, Anthony Gonzalez, Lee Bornstein, Ernesto Ablang, Tiffany Horton, Matthew Bosse, Paula Amezola, Ellen Rudy

Principal Investigator: Peter Kerndt

UARP Grant Number: ID04-LAC-028

Background: Recent advances in the treatment of human immunodeficiency virus (HIV) infection have created an urgent need for early HIV diagnosis and medical intervention. Currently available methods for HIV diagnosis employ antibody detection techniques that measure an individual's immune response to the presence of the virus. Variations in immune responses define a window period during which HIV antibodies are not detectable despite the presence of the virus. Persons receiving HIV testing through current methods may receive a negative result despite having HIV infection. Believing they are HIV-infection free, these persons with primary HIV may continue to engage in high-risk behaviors increasing the risk of onward HIV transmission during a period of maximal infectiousness. Detection of HIV infection in persons in the pre-seroconversion stage of infection is needed in order to facilitate referrals for early treatment, to prevent new infections from occurring and to expedite partner counseling and referral services.

Methods: Approximately 8,000 persons tested for HIV with a serum EIA and who are Ab negative will be tested using HIV viral load PCR RNA in pools up to 90 individual specimens. Positive pools will be deconstructed to identify an individual who may be primarily infected.

Expected Results: We estimate that pooled testing will yield one recent HIV infection per 500 to 1,000 HIV negative serum specimens (0.02% to 0.01%).

Conclusion: Data gathered from this project will be used to develop targeted testing strategies to identify primary HIV infection in high-risk populations and link this to early treatment referrals and partner counseling services.

Health Services Research and Quality of HIV/AIDS Care

Project IHRI: The Innovative Health Research Intervention

Presenter: William King, University of California, Los Angeles

Principal Investigator: Ronald Mitsuyasu

UARP Award Number: CH05-LA-608

Background: Hematopoietic stem cell therapy (HSCGT) is a new and evolving field of HIV therapy that is currently under preclinical and clinical investigation at UCLA. Historical, cultural and religious factors coupled with unfamiliarity can create significant misconceptions in the population about stem cell and gene therapy research, especially in low income and minority communities.

Goal: Innovative Health Research Intervention (IHRI) will be a psycho-educational intervention developed from an academic-community collaborative formed to improve stem cell research knowledge and its application to HIV therapy within low income and minority communities.

Methods: Project (IHRI) for HIV treatment will be a three phase study designed to increase knowledge about hematopoietic adult stem cell gene therapy (HSCGT) and to decrease medical mistrust regarding participation in novel clinical trials. Phase I will use qualitative research with 80 focus group participants to gather information regarding knowledge and current opinions about stem cell and gene therapy. This phase will incluye: (1.) six gender and ethnic specific focus groups of ten Black, White and Latino HIV positive males and females, 18 and older; (2.) one group of ten religious, civic and community leaders, and (3.) one group of ten health providers and community based organization (CBO) representatives. Phase II will use findings from Phase I to develop and pilot a three-session psycho-educational intervention—the Innovative Health Research Intervention (IHRI)—with 60 Black, White and Latino, HIV positive males and females. In this phase of the study, we can determine cultural appropriateness, feasibility, and acceptability of the overall intervention and its components. We will also assess the feasibility and acceptability of the assessment instrument. Phase III will be a randomized, controlled clinical trial to test the efficacy of the IHRI to a Standard HIV Attention Control intervention (ACI) with HIV positive Black, White and Latino males (n=360) and females (n=180) for a total sample of 540. Sessions will be led by trained treatment advocates at the four ethnically and culturally diverse NARLA sites: UCLA Center for Clinical AIDS Research and Education (CARE) Clinic, AIDS Project Los Angeles (APLA), To-Help-Everyone (THE) Clinic, and Friends Research, Inc./Van Ness Recovery House.

Expected Results: The intervention, IHRI, will be more effective in increasing knowledge, decreasing medical mistrust and improving willingness to participate in HSCGT clinical trials when compared to the control.

Conclusions: The findings from this study will help to better understand factors influencing the willingness of diverse populations of HIV infected individuals to participate in clinical trials of novel HIV therapeutic strategies such as HSCGT.

Progress: The protocol for Phase I is currently under review at the UCLA IRB. Investigators have contacted religious, community-based leaders and health care providers to become potential participants. Enrollment for Phase I will begin in early 2006.

Health Services Research and Quality of HIV/AIDS Care

Barriers to Enrollment in Research Registry for Microbicides Clinical Trials

Presenter: Steven Shoptaw, University of California, Los Angeles

Principal Investigator: Ronald Mitsuyasu

UARP Award Number: CH05-LA-608

Background: Rectal microbicides to inhibit the rectal transmission of sexually transmitted infections (STIs), including HIV, are in development. The public knows little about rectal microbicides. Clinical trials of rectal microbicides will require that the individuals likely to need and benefit from them be recruited into those trials. Given the sensitive nature of this novel approach to HIV prevention, specially designed materials will be required for their introduction both for clinical trials and beyond to optimize their value as a method of HIV prevention. We will develop educational materials about rectal microbicides, assess the best format in which to deliver such information and consider potential barriers to microbicide trial participation by analyzing factors that facilitate enrollment in a microbicide trial registry and retention in a cohort among men in Los Angeles from various racial/ethnic/gender/age/at risk-behavior groups.

Methods: In a longitudinal two-group experimental design, we will randomly assign 450 men (225 HIV negative and 225 HIV positive) into two conditions: (1.) a patient-centered video condition providing information describing rectal microbicides, their putative mechanisms of action, their potential efficacy, and side effects (n=225); or (2.) a standard of care condition that includes print materials covering the information contained in the video condition in the form of a brochure delivered by a study coordinator (n=225). These 450 participants will be recruited evenly at three the community and university sites including Friends/Van Ness, AIDS Project Los Angeles (APLA), and the UCLA CARE Clinic. All participants will be assessed four times: at baseline, post-intervention, three months and one year later by a web-based computerized interview.

Results: We will assess the various features of microbicides that may affect high risk men's willingness to participate in research and adopt use of microbicides for HIV prevention. Videotapes versus staff-delivered educational interventions will be developed, validated and administered to these individuals at the first and subsequent follow up interviews. APLA will lead the development and evaluation of educational materials and their evaluation for this study. We will identify which type of material is more valid for informing men about rectal microbicides. We will also assess retention rates at follow-up among different risk groups and factors affecting loss to follow up, any changes in attitudes and sexual behavior between time points, and we will establish a registry of all interested participants for potential inclusion in future clinical trial(s) of rectal microbicides.

Conclusion: This project will contribute to the overall goal of NARLA and enhance the capacity of UCLA and its community partners to collaboratively conduct clinical trials on important new methods of HIV prevention. It will also collect information needed prior to conduct of an actual clinical trial on rectal microbicides.

Health Services Research and Quality of HIV/AIDS Care

Impact of Comorbid Illness on Persons Living with HIV/AIDS

Presenter: David Zingmond, University of California, Los Angeles

Collaborators: William E. Cunningham, Judith Currier

Principal Investigator: David Zingmond

UARP Award Number: ID05-LA-034

Background: The current model of care for HIV may not reflect the changing needs of the HIV-infected population. Complications arising from non-HIV comorbid illness are significant and increasing sources of disease for persons living HIV infection. Side effects of medication, aging of the population and other factors have contributed towards increasing comorbid illness (e.g. diabetes, coronary artery disease, hypertension, hepatitis C infection, osteoporosis, and psychiatric disorders). In the general population, these conditions are known to be associated with greater healthcare utilization, morbidity and mortality. Little is known regarding trends in the occurrence or impact of non-HIV comorbid illnesses on care and outcomes in HIV-infected populations in the U.S. This is clinically important to California, which has the second largest number of HIV-infected persons in the U.S., and also at the policy level because of the large number of HIV-infected individuals receiving care through the public healthcare system. This research will address both of these issues, as well as an evaluation of one particular aspect of caring for comorbid illness – use of specialty care. This research will: (1.) examine the rate of non-HIV comorbid illness in persons living with HIV, (.2.) explore the impact of non-HIV comorbid illness on outcomes in persons living with HIV, and (3.) evaluate the use of specialty care for non-HIV comorbid illness and its clinical outcomes – disease-specific events and mortality.

Methods: The data to be used in the study will be a detailed research database linking established state databases to evaluate these aspects of non-HIV comorbid illness in the California Medicaid (Medi-Cal) population, a diverse population with monthly enrollment exceeding 23,000 persons. Medi-Cal pays for the majority of AIDS-related hospitalizations in California and is the single largest payer of care for HIV-infected persons in California. The Medi-Cal population can serve as a sentinel population for all HIV-infected persons in the state – understanding their care needs will inform the care needs for persons receiving care through other mechanisms.

Results: We will identify rates of non-HIV comorbid illness in HIV-infected Medi-Cal enrollees, the impact of these diseases on patient outcomes and the effect of access to care non-HIV generalist and specialty care on disease-specific events and outcomes.

Conclusions: Improving the quality and outcomes of persons living with HIV infection is a dynamic process and this study will provide a framework for describing the HIV/AIDS population receiving care under public financing and identifying the most important needs based upon identified non-HIV-related illnesses that contribute towards patient outcomes. Results of this study will inform clinical and policy decisions and will provide a platform for further in-depth studies to expand our understanding of the quality of care delivered to HIV-infected persons in California.

Health Services Research and Quality of HIV/AIDS Care

Community-Acquired Methicillin Resistant Staphylococcus aureus Colonization among HIV-Infected Persons

Presenter: Loren Miller, LA Biomedical Research Institute at Harbor-UCLA Medical Center

Collaborators: Eric S. Daar, Mallory D. Witt, Mario Guerrero, Jennifer Tan, Gunter Rieg

Principal Investigator: Loren Miller

UARP Award Number: ID04-REI-045

Background: Outbreaks of community-acquired methicillin resistant Staphylococcus aureus (CA-MRSA) among HIV-infected men who have sex with men (MSM) have been increasingly reported. In many HIV clinics, including ours, the majority of skin and soft tissue infections are now caused by CA-MRSA. Nasal MRSA colonization is known to be a risk factor for MRSA infection in hospitalized patients. However, the role of S. aureus nasal colonization in the pathogenesis of non-hospitalized CA-MRSA remains poorly understood. We hypothesized that nasal colonization with CA-MRSA would exceed that of methicillin-susceptible S. aureus (MSSA), akin to observations that the incidence of clinical disease from CA-MRSA exceeds that of CA-MSSA in our population. To test this hypothesis, we performed a survey of S. aureus nasal colonization among HIV-infected persons.

Methods: We prospectively surveyed HIV-infected MSM followed in the Harbor-UCLA Medical Center HIV clinic. After informed consent was obtained, patients were administered a questionnaire on CA-MRSA risk factors and a nasal swab for S. aureus was performed.

Results: Of 309 enrolled subjects, 78 (25%) had nasal colonization with S. aureus. Of these, 61 (78%) were colonized with MSSA and 17 (22%) with MRSA. MRSA colonized subjects were more likely than those not colonized with MRSA to report a skin infection in the past six months (53% v 18%, RR 3.0 [95% CI 1.8 – 5.0], p=0.0004) and to have close contact with a person with a skin infection in the past six months (21% v 5%, RR 4.3 [95% CI 1.4 – 13.2], p=0.04). There was a non-significant trend towards persons with MRSA colonization having high risk sexual behavior compared to non-colonized patients (18% versus 6%, RR 2.9 [95% CI 0.95 – 9.1], p=0.09). There was no relationship between MRSA colonization and: gender, MSM, recent hospitalization, residence in a chronic care facility, self-reported drug use, recent incarceration or anonymous sex while using drugs or alcohol. No factors were associated with MSSA colonization except lack of anonymous sex while using drugs or alcohol (RR 0.22 [95% CI 0.06 – 0.88], p=0.01).

Conclusion: Although most skin and soft tissue infections in our clinic population are caused by MRSA, the majority of S. aureus nasal colonization is with MSSA. Additionally, MRSA colonization is associated with previous skin infection or contact with persons with skin infections, while MSSA colonization is not associated with these factors. Our findings suggest that the infectivity and transmissibility of nasal CA-MRSA may be higher than nasal CA-MSSA strains. Longitudinal studies are needed to clarify this relationship.

Compendium Only

Prevalence and Determinants of Late HIV Diagnosis among HIV+ Individuals in Tijuana, Mexico

Principal Investigator: Melbourne Hovell, San Diego State University

Collaborators: Claudia M. Carrizosa, Ana Martinez-Donate, Carol Sipan, Elaine Blumberg

UARP Award Number: IS02-CBECH-711

Background: In California, Latinos represent a large and growing percentage of people diagnosed with HIV/AIDS. More than one-third of HIV positive Latinos present late with AIDS diagnosis. Anecdotal information from Tijuana's local health professionals suggests that people living with HIV/AIDS in this Mexican border city are also very likely to present with advanced AIDS disease without a prior HIV diagnosis. Given that high risk behaviors occur between residents of the Mexican northern border region and California, late HIV testing and diagnosis in Tijuana may contribute to higher transmission rates and result in missed opportunities for prevention and effective treatment of HIV in the California-Mexico border region.

Purpose: This study will examine the prevalence and determinants of late HIV diagnosis among adults living in Tijuana served by the two public HIV clinics operating in this city: IMSS and ISESALUD. t is estimated that these clinics serve approximately 80% of the people living with HIV/AIDS in Tijuana.

Methods: Medical charts for all HIV/AIDS cases archived in these two clinics will be reviewed for abstraction of demographic and clinical data. Structured interviews are being conducted with 360 clients seeking HIV care in the two study clinics. Social and environmental determinants for late HIV diagnosis and risk behaviors for HIV transmission and reinfection will be explored based on our Behavioral Ecological Model.

Results: Preliminary analysis of a sample of 70 medical charts indicates that close to half (46%) presented undiagnosed with advanced AIDS disease based on clinical conditions and CD4 counts (< 200 cells/mm3). Preliminary analysis of 228 interviews (149 men and 79 women) indicates that 85% of the sample has immigrated to Tijuana from other Mexican regions and 25% have a history of residence in the U.S. Among male participants, 37% self-identified as homosexual or bisexual. Among females, only one self-identified as bisexual. Sexual transmission was the reported mode of HIV acquisition in 81% of all cases, with nearly half (45%) attributed to sex with a casual partner. Reported barriers to HIV testing include: not feeling sick (74%), not feeling at risk (58%), fear of disclosure to others (53%), thinking that the test was expensive (34%), and lacking money for HIV testing (26%). Half of the sample (53%) never thought about getting tested for HIV or had an HIV test (55%) before testing positive. Triggers of HIV testing included: doctor's recommendation (63%), feeling at risk (49%), and being hospitalized (25%).

Conclusions: Data collection will be completed by the end of 2005. Descriptive statistics and results from estimated predictive models of late HIV diagnosis resulting from data analysis with the whole sample will be presented. Study findings will inform interventions to increase early HIV diagnosis and decrease HIV risk behaviors among HIV positive individuals. These interventions are imperative to improve the prognosis of individuals infected with HIV and to slow the transmission of HIV infection in the California-Mexico border region.

Compendium Only

Traumatic Event History and PTSD Symptoms in Vulnerable Populations Receiving Primary Care for HIV/AIDS in a Public Healthcare Setting

Principal Investigator: Cheryl Koopman, Stanford University

Collaborators: D. Prentiss, Dennis Israelski, G. Balmas, S. Cummings, J. Castello

UARP Award Number: CR01-ST-090

Background: Considerable evidence suggests that people with HIV are significantly more distressed than the general population. This paper examines the prevalence of traumatic events and symptom criteria for post-traumatic stress disorder (PTSD).

Methods: From approximately 350 patients attending two County-based HIV primary care clinics, 210 participants were screened for diagnostic symptom criteria for PTSD for the cross-sectional survey. Participants were primarily male (71%) and the mean age was 42 years (s.d. = 8.7). Ethnic breakdown of the sample was: 34% African-American, 33% Latino, 26% Caucasian, and 7% other or mixed ethnicity. Participant interviews elicited reports of traumatic events using the Trauma History Questionnaire (7-92%). Prevalence of diagnostic symptom criteria for PTSD was 34%.

Results: We examine: (1.) prevalence of reported events in several categories of traumatic events including: crime and violence, physical and sexual abuse, personal loss, and general disaster, (2.) differences in types of traumatic events experienced due to sex and ethnicity, (3.) prevalence of traumatic experiences beginning in childhood, (4.) traumatic events and PTSD symptom outcomes, (5.) the relationship between categories of traumatic events and PTSD, depression and ASD, and (6.) the relationship between traumatic events which occurred in childhood and PTSD, depression and ASD.

Conclusions: Multiplicity of traumatic events does not reflect the traditional model of PTSD. Clinical Implications: screen certain patients in primary care settings, screen all patients in HIV primary care settings and abandon single trauma mentality.

HIV and Prevention 3

HIV and African Americans: Social and Behavioral Risk Factors

Coping with the Consequences of What Happens Inside Prison: Preliminary Findings from the Formative Phase of Project Holla

Presenter: Barry Zack, University of California, San Francisco

Collaborators: Craig Hutchinson

Principal Investigators: Janet Myers, Barry Zack

UARP Award Number: AL04-SF-806

Background: Little is known about the best ways to tailor HIV/STD/hepatitis prevention and treatment services in light of personal and community-based issues facing men after they leave prison. It is not known whether men who engage in sex with other men or needle sharing while in prison continue these behaviors when they reenter the community. Furthermore, we do not know if men engage in HIV prevention behaviors, or the extent to which they seek HIV testing and medical care. In Project Holla, we are exploring the context of HIV risk behavior among men leaving prison and aim to design an intervention based on men's input and experiences so that it can address the unique HIV prevention needs of recently released men.

Methods: In the formative phase of this multi-phase study, we recruited men who were recently released from prison and conducted serial in-depth qualitative interviews to develop an understanding of their experiences and their community. For the next phase of the project, we plan to use the information gained through the qualitative interviews as a guide to choosing, tailoring, implementing and evaluating an intervention to increase HIV testing and decrease risk behavior. We plan to examine short-term trends in HIV testing and HIV risk behavior outside of prison and associations between testing, risk and exposure to the intervention among men in the study. In addition to the quantitative survey, we will conduct qualitative interviews to explore facilitators and barriers to intervention effectiveness.

Results: We are presenting results from the formative phase of the project, during which we conducted over 50 hours of in-depth interviews with men recently released from prison. We interviewed 28 men, 25 of whom were interviewed twice. All but one was African American. Men had been released from diverse corrections settings across the state and described an array of strategies for coping with threats to health while inside prison. Most felt that their families and friends understood what they had been through. Although they did not typically volunteer information, they were willing to discuss incarceration experiences if asked directly by sex partners, family or friends. Very few men described their own sexual contact with other men inside prison but all were able to relate stories regarding the extent and social dynamics of prison sex and drug use inside. With an exception for some men who were out as "gay," men said that there was little tolerance for sex among men and that when it did occur, it was considered a shameful act. Many remarked that the prevalence of sex inside prison was sensationalized in popular culture. However, all men were concerned about HIV, most had taken steps to avoid infection inside, and many had discussed HIV with their sex partners on the outside. Men described a need for HIV testing and other services and indicated that what happened to them inside influenced their need for services after release.

Conclusion: Although these results are preliminary, they are helping us understand the context of HIV/STD/hepatitis risk for men leaving prison, describe the consequences of behavior on the inside for behavior on the outside, and identify important issues to consider in designing and delivering services to men leaving prison.

HIV and African Americans: Social and Behavioral Risk Factors

HIV Prevention for Men on the Down Low

Presenter: Carla Dillard-Smith, California Prevention and Education Project (CAL-PEP)

Collaborators: Perry Rhodes III, Don Operario

Principal Investigators: Carla Dillard-Smith, Susan Kegeles

UARP Award Number: AL04-CAPEP-817

Introduction: California Prevention and Education Project (CAL-PEP) has participated in community-based research for high-risk populations in Oakland and San Francisco, California for thirteen years. Both cities are major HIV epicenters. African American men who have sex with men have been highly impacted by HIV, with reports showing disproportionate levels of HIV infection, HIV-related illnesses and mortality rates due to AIDS among these men.

Several research studies indicate that many African American MSM (Men who have Sex with Men) do not identify themselves as gay or bisexual and may not perceive themselves at risk for HIV, undergo HIV testing, utilize HIV prevention services, or disclose their sexual behaviors to others. CAL-PEP has long provided services to African American men who have sex with men who do not identify as gay and bisexual.

Intervention: With a grant from the Universitywide AIDS Research Project, CAL-PEP is collaborating with the Center for AIDS Prevention Studies. The HIV prevention and health promotion intervention is designed specifically for African American MSM who do not identify as gay or bisexual. We have enhanced the traditional HIV interventions by combining confidential HIV counseling and testing with a series of individualized health promotion counseling sessions. The intervention will also hold groups where men who chose will have the chance to discuss issues of interest in an informal environment. HIV prevention will also be addressed.

Method: A sample of 200 African American MSM who do not identify as gay or bisexual will be recruited from Oakland and San Francisco. After receiving HIV counseling and testing, half will be randomly assigned to the enhanced intervention condition.

Discussion: Creating effective HIV interventions for African American men who have sex with men (MSM) is among the most urgent priorities for public health. Through formative research and input from members of this group and community gatekeepers, this project will develop a culturally and gender-appropriate intervention tailored to the needs of these men. The proposed study will compare the enhanced HIV intervention, which involves HIV counseling and testing plus a series of individual health promotion counseling sessions, relative to a standard program involving HIV counseling, testing and referral to case management services. This research aims to build on prior literature and fill important gaps in HIV prevention for African American MSM who do not identify as gay or bisexual. We will use qualitative techniques to develop more sophisticated insight into the diversity of African American DL (Down Low) men and their lives. Building upon this knowledge, we will finalize the procedures for a theory-driven HIV prevention intervention for DL men. We will conduct process and outcome evaluation to describe the feasibility and acceptability of this intervention. Finally, we will disseminate experiences and evaluation findings to professional, community, and scientific networks, and use findings to develop further strategies for HIV prevention for African American men.

HIV and African Americans: Social and Behavioral Risk Factors

Attitudes towards HIV Transmission, Condom Use, and Disclosure among HIV-Positive Non-Gay-Identifying African American Men

Presenter: Nina Harawa, Los Angeles County Sheriff's Department

Collaborators: John K. Williams, Hema C. Ramamurthi, Sergio Avina, Cleo Manago, Tony Wafford, Kevin Pickett

Principal Investigator: Nina Harawa

UARP Award Number: AL04-LASD-840A

Background: HIV/AIDS rates continue to be disproportionately high among homosexually and bisexually active men, especially those who are African American. Recent attention to the high levels of HIV infection among African American men who have sex with men or both men and women (MSM/W) and the frequent non-gay-identification in this population highlight the importance of understanding how HIV-positive African American MSM/W perceive safer sex, experience living with HIV, and decide when to disclose their HIV status.

Methods: In June 2005, three focus groups were conducted with 30 predominately HIV-positive and non-gay identifying African American MSM/W. Participants were recruited from Los Angeles County through collaborating community-based organizations, posted fliers, and word of mouth. Using a constant comparison methodology we examined responses to focus group questions regarding perceptions of condom use and the effect of being HIV positive on sexual activity and serostatus disclosure.

Results: The major themes regarding condom use included its protective role against disease and pregnancy, condom acceptability including aesthetics factors and concerns about effectiveness, and situational pressures including exchange sex, substance use, and potential questioning from female partners. Themes pertaining to the impact of HIV infection on the sexual lives of these men included isolation, perceived rejection, and decreased partner seeking. Themes regarding disclosure of HIV status included selective disclosure and the responsibility to disclose. These themes influenced some men to disclose and to practice safer sex and others to forego condoms and disclosure or avoid the issue altogether by not seeking out regular sex partners.

Conclusions: Non-gay-identified African American MSM/W must weigh a complicated set of risks and benefits associated with safer sex and HIV disclosure to sexual partners. These concerns, together with condom acceptability issues and situational factors that reduce individual control over condom use, may interfere with a desire to protect others from HIV infection or oneself from other sexually transmitted infections and HIV reinfection. The results of this analysis will inform curriculum development for a UARP-funded HIV prevention intervention for non-gay-identified African American MSM/W.

HIV and African Americans: Social and Behavioral Risk Factors

HIV Outreach and Testing with Non-gay Identified African American MSM

Presenter: Edward Mamary, San Jose State University

Collaborator: Jacqueline Siller

Principal Investigator: Edward Mamary

UARP Award Number: ID04-SJSU-037

Background: African American men who have sex with men (MSM) and who do not identify as gay are at high risk for HIV infection. Many African American MSM do not seek HIV testing, despite engaging in behaviors that place them at risk for infection. Previous strategies have proven to be ineffective in reaching non-gay identified (NGI) African American (MSM) who do not fit into traditionally defined gay or bisexual categories. Many NGI African Americans cannot relate to HIV prevention messages that are designed for the gay community; furthermore, many do not read gay print media, and few participate in gay identified events or patronize gay establishments. This study will inform prevention efforts by exploring the motivations, capacities, and challenges associated with HIV prevention among this hard-to-reach population.

Methods: A two-tiered qualitative study design will be used to gather data to examine the perceptions of NGI African American MSM in the San Francisco Bay Area regarding their HIV risk and the social, cultural, community, and family influences associated with conducting community outreach, HIV testing, and prevention. During the first phase of the project, 25 in-depth qualitative interviews will be conducted. During the second project phase, 12 individuals from the initial sample will be invited to participate in Photovoice, an innovative participatory action research strategy that uses a photographic technique to promote critical reflection by research participants regarding the phenomenon of interest. This method will explore further the perceived strengths and concerns related to HIV risk of participants.

Expected Results: As of November 2005, we have conducted in-depth interviews with 19 men who fit the inclusion criteria of the study. Preliminary results have revealed a few notable observations that have relevance for culturally relevant HIV prevention efforts. Women have been identified as major supports in the lives of many of the men and may serve as valuable resources for HIV prevention efforts. Despite the fact that many men have characterized the church as opposing same sex behavior, many still consider themselves to be observant Christians. For most, if not all of the men, the concept of harm reduction has not been discussed as an HIV prevention strategy. The Photovoice phase is scheduled to begin in the second year of the study. Most of the 19 men interviewed have expressed interest in participating in the Photovoice component of the project. The outcomes of this participatory action research are expected to inform policies and programs related to HIV prevention, testing, counseling, and outreach to African American NGI-MSM. The collection of visual images may provide data for use in innovative community awareness campaigns. Documenting innovative outreach methods will also assist other researchers in formulating their sampling strategies with this hard-to-reach population.

HIV and African Americans: Social and Behavioral Risk Factors

Community Input for Effective HIV Prevention for African Immigrants in California

Presenter: Yewoubdar Beyene, University of California, San Francisco

Principal Investigator: Yewoubdar Beyene

UARP Award Number: ID05-SF-005

Background: Immigrants from the continent of Africa are the most recent immigrant population in the US and steadily growing. California hosts the greatest number of these immigrants. The majority of these immigrants come from sub-Saharan Africa where over two-thirds of all people are now living with HIV. Reports from community clinics and state health data suggest that HIV/AIDS rate of infection and risk behavior are significant problems in the African immigrant communities. Despite these realities, HIV intervention efforts have largely overlooked African immigrant communities. Currently, there are no culturally informed educational interventions targeting African immigrants in California.

Methods: Using focus group interviews with African immigrant communities' key informants, the goals of this feasibility study are 1) to explore cultural response to an existing basic HIV transmission prevention training module and the acceptability of the format and wording of the various topics addressed and 2) integrate community input into the intervention module to develop a culturally informed educational intervention that can be used by African immigrant communities within California.

Expected Results: This study takes the first steps in developing culturally acceptable HIV educational intervention for African immigrants. The resulting module will be further tested in future studies addressing HIV education in this population.

HIV and African Americans: Social and Behavioral Risk Factors

HIV Translation Research for Young African American MSM

Presenter: Susan Kegeles, University of California, San Francisco

Collaborator: Greg Rebchook

Principal Investigator: Susan Kegeles

UARP Award Number: TR02-SF-510A

Background: Young Black men who have sex with men (YBMSM) are at extremely high risk for HIV both in California and throughout the U.S. The Mpowerment Project (MP), an innovative, community-level HIV prevention program for young MSM, ages 18-29, has been shown through randomized, controlled trials to reduce rates of unprotected sex. It is based on community and individual empowerment, community organizing, and peer outreach principles. Many Black CBOs are interested in the MP partly because its guiding principles support an "Afrocentric worldview," valuing the community, unity, self-determination, cooperation, mutual responsibility, and spirituality. However, these CBOs know that the MP must be adapted and tailored in order to be effective with YBMSM.

Methods: In order to determine how to tailor MP, we convened two Boards of Cultural Experts (BOCEs) in Oakland and Los Angeles. The BOCEs were primarily comprised of Black adult MSM knowledgeable about Black MSM issues (n=21). The BOCEs met 8 times to "deconstruct" MP regarding its appropriateness and utility for YBMSM, and they reviewed all aspects of the Mpowerment Project and offered views of how to modify it to reach YBMSM. We also conducted focus groups with YBMSM in Oakland and Los Angeles to examine specific components of the intervention. Themes that arose in the various groups were analyzed and implications for changes to MP were developed. BOCEs approved proposed adaptations. After completion of the formative research phase, the Sexual Minority Alliance of Alameda County began pilot-testing the new Mpowerment Project with YBMSM in Oakland, CA. Additionally, the Minority AIDS Project and the Black AIDS Institute collaborated on a pilot-test of the tailored intervention in Los Angeles. To support the CBO efforts, CAPS provided an intensive 3-day training, program materials, on-going technical assistance, and access to web-based resources. CAPS also studied the success of the implementation process.

Results: Formative research revealed that MP's guiding principles (e.g., community-building, empowerment, peer outreach), structure (paid staff, volunteers, core group) and intervention components (small groups, publicity, formal outreach, informal outreach, community space) continue to be relevant for YBMSM. However, many issues arose regarding the need for substantial adaptation of the intervention. Our presentation will discuss how MP needs to be adapted in order to address themes such as: internalized oppression, "whole man" issues, diverse sexual identities, lack of adult male role-models, discomfort with traditional pedagogy, and church/religion/spirituality. We will also address the process of implementing the tailored intervention. Before the programs could begin many issues needed to be addressed, including: identifying and training staff; conducting community assessments; determining supervision and administration processes; developing data collection plans; locating, securing and decorating space; and establishing credibility with the community.

Conclusions: The BOCEs and focus groups concluded that MP's outline was culturally appropriate, but how it is implemented needs to be changed, and it must also address many other issues important to YBMSM. Implementing the tailored intervention was a complex and time-consuming process. In addition to presenting our formative conclusions and summaries of each program's process data, we will also address the major implementation challenges and barriers that arose, and we will outline a research agenda that is necessary in order to fully develop an intervention prototype that other Black organizations across the state and country can implement in their communities.

HIV and African Americans: Social and Behavioral Risk Factors

HIV/AIDS Conspiracy Theories: Barriers to HIV Prevention?

Presenter: Sonja Mackenzie, University of California, Berkeley

Principal Investigator: Sonja Mackenzie

UARP Award Number: D05-B-405

Background: Conspiracy theories have accompanied the emergence of plagues from the Middle Ages to the present day. The types of conspiracy theories associated with HIV/AIDS range from theories involving government involvement in the creation of the virus to beliefs that testing and medications themselves can be used as instruments to wipe out "undesirable" populations. HIV/AIDS conspiracy theories place HIV prevention in the context of the historical, economic and cultural context in which people become vulnerable to HIV. Conspiracy theories speak to the structural determinants of HIV and, therefore, suggest structural-level interventions to address disparities in HIV/AIDS, in contrast to the majority of HIV prevention efforts that focus on individual behaviors. In California, African Americans comprised 7% of the general population yet 21% of all reported HIV cases from 2002 – 2003. In Alameda County, African Americans are similarly overrepresented among AIDS cases as compared to their proportion of the general population -- African American men and women constitute 15% of the general population, yet 51% of reported AIDS cases in 2002-2003. These trends indicate that current HIV prevention efforts in California are having limited success in decreasing the rates of HIV infection among African American men and women. Research on HIV/AIDS conspiracy theories has consistently found that approximately one quarter of African Americans of all socioeconomic backgrounds hold conspiracy beliefs, and has concluded that they present significant challenges to HIV prevention. HIV/AIDS conspiracy theories have been hypothesized as barriers to HIV testing among African Americans; as barriers to health education and health care; as barriers to needle exchange; and as barriers to participation in research. Recent research has found stronger conspiracy beliefs among men. This study aims to contribute to the development of HIV prevention measures for African Americans by examining the ways that HIV/AIDS conspiracy theories might present barriers to HIV prevention efforts with African Americans, and by exploring gender differences in these theories.

Methods: This research presents preliminary qualitative work of a larger mixed-method dissertation project on HIV/AIDS conspiracy theories. This research will investigate through in-depth qualitative interviews the types, meaning and implications of HIV/AIDS conspiracy theories among a sample of HIV- and HIV+ African American men and women (n=40), as well as among a sample of 10 HIV prevention providers. Study participants will be recruited using targeted street-based and venue-based convenience sampling. Qualitative interviews will allow this study to generate in-depth narratives regarding if and how HIV/AIDS conspiracy narratives resonate for African American men and women; which conspiracy theories might be particularly meaningful; and a consideration of the implications of these theories on service use and on risk behaviors. The combination of interviews with community members and providers aims to generate a dataset of interviews from two key perspectives to inform prevention practice and future research.

Expected Results: At this time, a Community Meeting has been held with HIV prevention providers to launch the project. This community dialogue generated rich formative data vital to the development of this project. Pilot interviews will be conducted in December 2005. Preliminary data from the Community Meeting and from initial analysis of interviews will be presented at the UARP Annual Meeting. However, given the initial stage of this research, these data will be oriented towards the process of the research rather than considered as outcome data.

HIV and Latinos: Social and Behavioral Risk Factors

Multi-level Prevention in Culture and Context with Latino MSM

Presenter: LeRoy Blea, City of Berkeley, Health and Human Services Dept.

Collaborator: Diane Binson

Principal Investigator: LeRoy Blea

UARP Award Number: AL04-BHHS-809

Men who have sex with men (MSM) have been, and continue to be, one of the demographic groups most disproportionately affected by HIV in the United States, especially in California. The impact of HIV disease on Latinos in the U.S. is particularly severe, as Latinos account for 19% of cumulative AIDS cases while representing only 13% of the population. In Berkeley, census data show that Latinos comprise 10% of the population, yet 18% of the HIV positives identified at publicly funded HIV test sites in the City. Over 70% of these are MSM. It is critical to address the growing epidemic among Latino MSM in a way that addresses the considerable challenges of providing HIV prevention services for this population. The planned project will develop, implement, and evaluate an intervention for Latino MSM who do not self-identify as gay (MSM/NG). The project will rely on an established community collaborative relationship between the University of California San Francisco and the City of Berkeley AIDS Office. The evaluation component will use tested measures and methods from previous and current studies of the project team. The specific aims for the planned project are to:1. Design an intervention in dialogue with the target community of Latino MSM/NG to ensure a culturally sensitive, multi-level intervention that will address critical aspects of HIV prevention both at the individual and structural levels in public sex environments (PSEs).2. Implement the intervention at the individual and structural levels by providing a 1-on-1 intensive non-traditional counseling and testing session using lotteria cards, or photonovellas, or peer stories that contain coded community-derived themes that may include racism, homophobia and other risks particular to Latino MSM; and by carrying these themes to the context of sexual behavior in a social marketing campaign that will lead men to continue an internal and community dialogue about risk and risk behavior. In addition, we will pursue changes in the context of sexual behaviors to improve lighting, condom availability, and policy changes that increase safety for Latino MSM when having sex in these venues.3. Conduct pre-post outcome and process evaluations of the intervention using a pre-post survey methodology for the outcome evaluation with baseline assessment of 150 men just prior to their participation in the intervention and a follow-up interview conducted 3 months later; and process evaluations using focus group and in-depth interview methods.

HIV and Latinos: Social and Behavioral Risk Factors

Social Marketing to Reduce HIV Risk among Non-Gay Identified Latino Men Who Have Sex with Men

Presenter: Ana Martinez-Donate, San Diego State University Research Foundation

Collaborators: Fernando Sañudo, Jennifer Zellner, Melbourne Hovell, Carol Sipan, Araceli Fernández, Moshe Engelberg

Principal Investigator: Ana Martinez-Donate

UARP Award Number: AL04-SDSUF-804

Anecdotal reports indicate that non-gay identified (NGI) Latino men who have sex with men (MSM) are isolated and secretive about their sexual behavior, and are not being reached by existing HIV prevention programs targeting Latino MSM. Thus, this population may be at increased risk for HIV and other sexually transmitted infections (STI). Vista Community Clinic (VCC) seeks to access NGI Latino MSM by providing a free male health exam that assesses general physical health regardless of sexual practices, and includes HIV and STI testing as well as non-judgmental individual risk reduction counseling and referrals. The purpose of this project is to test the effectiveness of a social marketing campaign to promote condom use, HIV testing, and participation in VCC's risk reduction activities, including the male health exam, among NGI Latino MSM in North San Diego County. Completed formative research activities include (a) 24 one-on-one key informant interviews, including 12 with NGI Latino MSM, 6 with employees of venues frequented by NGI Latino MSM, and 6 with Latina women who are family members, friends, or current or former significant others of NGIs.; (b) two focus groups, one conducted with key gay-identified Latinos who prefer NGI MSM as sexual partners, and the other conducted with VCC staff who have NGI Latino MSM clients; and (c) field visits, direct observations, and head counts conducted at potential intervention sites. Analyses of qualitative data from key informant interviews and focus groups are currently under way. Significant preliminary themes that have emerged include cultural issues such as the importance of family, religion, and masculinity; the association of condom use with distance between partners, promiscuity, and infidelity; lack of condom use on the basis of a clean or healthy looking partner; and the use of alcohol as a justification for sexual encounters with other men. These and other results will inform the design of preventative materials and activities for the social marketing campaign. In addition, observations and head counts have helped us identify 12 sites (5 high risk and 7 low risk venues) in which we plan to implement campaign activities and conduct surveys to evaluate its effectiveness. Beginning in December 2005, bi-monthly baseline intercept surveys with independent samples will be implemented at both high-risk and low risk venues (N = 230 per survey wave) to assess baseline target behaviors, attitudes, and use of clinic services. Preliminary baseline results will be presented. Future plans include the implementation of the social marketing campaign in April 2006, accompanied by continued bi-monthly intercept surveys to assess exposure to the campaign and changes in target behaviors, attitudes, and use of clinic services. The campaign will remain in place through November 2006, at which time all campaign activities will cease. Bi-monthly intercept surveys will continue to be conducted through May 2007. The results of this project will inform the effectiveness of our social marketing intervention to promote safe sex behavior and enhance health care utilization among Latino males in general and NGI Latino MSM in particular. This study will also explore whether these materials and activities contribute to reduced social stigma, increased social acceptance of individuals who engage in same-sex practices, and community mobilization to promote and reinforce preventive behaviors. Study findings may inform future campaigns to reduce HIV risk among the Latino population in California.

HIV and Latinos: Social and Behavioral Risk Factors

Latino Day Laborers' HIV Risk in Targeted Geographical Areas

Presenter: Frank Galvan, Charles R. Drew University of Medicine & Science

Collaborators: Daniel Ortiz, Anthony Moreno, Victor Martinez

Principal Investigator: Frank Galvan

UARP Award Number: ID04-DREW-023

Background: An understudied group for HIV risk is the population of Latino male immigrant day laborers. Information suggests that some day laborers are initially hired for paid labor and then solicited for homosexual activities. Some specific day laborer sites are known to be frequented by day laborers engaging in paid homosexual activity or are identified by internet websites as catering to men who are interested in day laborers for sexual activity. This study sought to identify the extent to which day laborers at specific targeted sites in Los Angeles County are potentially at risk for HIV because of their work as day laborers and the extent to which they are engaging in HIV-related risk behaviors.

Methods: Four hundred and fifty day laborers were recruited at specific geographical locations known to be frequented by day laborers engaged in paid homosexual activity or were identified by internet websites as catering to men who specifically are interested in finding male Latino immigrant day laborers for paid or unpaid sexual activity. Participants were over 18 and under 40 years of age. All participants were verbally administered a survey. In addition, 35 of these individuals also participated in a more in-depth qualitative interview.

Results: All data have already been collected. The entry of the quantitative data into a statistical program for subsequent analysis has just recently been completed. The qualitative data are still being transcribed. Preliminary analysis has begun with the quantitative data; no analyses have been conducted yet with the qualitative data. The overwhelming majority of the day laborers (89%) reported being undocumented residents of the U.S. Preliminary results reveal that 38% of the day laborers reported having been approached for sex by a man while seeking work as a day laborer in California or after being contracted for work. Of those reporting this having happened, the number of times that this was said to have occurred ranged from 1 time to 100 times. The most reported number of times were twice (23%), once (22%), three times (20%), five times (9%) and 10 times (7%). Of those reporting having been approached, the overwhelming majority (91%) reported never engaging in sex with the individuals who approached them. Only 9% reported having had sex, with approximately equal numbers reporting having had sex "always," "usually" and "sometimes" with their solicitors . Most of the individuals who did have sex (88%) reported having been paid for having sex at least sometimes. Among those that did get paid, all of them (100%) stated that they accepted being paid for sex because they needed the money. Most of those that accepted payment (86%) stated that it was because they had not had any work all day long. Although only 14% of those that accepted payment reported being gay or bisexual themselves, 29% stated as a reason for accepting payment that they enjoyed having sex with other men. All of those who reported having sex reported having received oral sex at least sometimes. Only a quarter reported having given oral sex. Eighty-eight percent reported having had penetrative anal sex at least sometimes, and of these only 29% reported always using a condom. Only 6% (1 person) reported having had unprotected receptive anal sex. Fifty-one percent of all 450 day laborers reported having ever tested for HIV. Only 1 person reported being HIV-positive.

Conclusion: An unexpectedly high number of day laborers report having been approached for sex as part of their day labor activities. Most do not subsequently engage in sexual activities with their solicitors. However, among those having sex, a very large percent report unsafe penetrative anal sex. Further HIV prevention programs should be directed to this population.

HIV and Latinos: Social and Behavioral Risk Factors

Health Care Access among Mexico-California Migrants: Evidence from the California-Mexico Epidemiological Surveillance Pilot

Presenter: Alvaro Garza, University of California, San Francisco (Fresno)

Principal Investigator: Alvaro Garza

UARP Award Number: CM02-SF-806

Background/Objectives: To describe the utilization of selected preventive healthcare services, and identify demographic, social, and behavioral characteristics associated with receiving services among Mexican migrants in California.

Methods: We analyzed data collected between January and December 2004 in Fresno and San Diego Counties, California, for the California-Mexico Epidemiological Surveillance Pilot, a cross-sectional survey composed of a venue and housing-based, targeted random sample of Mexico-California migrants using a 35-minute face-to-face questionnaire. Acculturation was measured by seven-questions on language preference scored in Likert format. HIV transmission-risk knowledge was measured by the percent correct of nine true/false questions. We compared characteristics between migrants who received vs. not received health services.

Results: Of 792 respondents, 336 (42.4%) received health services for HIV/AIDS, STI, or TB within the past 12 months. Approximately two-thirds received services in California. Compared to migrants who did not health receive services, those who received services had more years of education (9 vs. 7 years; $P<0.001$), and a greater prevalence of schooling in the U.S. (18% vs. 8%; $P<0.001$), a high/medium language acculturation score (74% vs. 49%; $P<0.001$), health fair attendance in the last year (26% vs. 14%; $p<0.001$), access to condoms when needed (47% vs. 34%; $P<0.001$), and a higher HIV transmission-risk knowledge score (82% vs. 77%; $P<0.001$).

Conclusions: The Mexico-California migrant population in Fresno and San Diego Counties primarily receive HIV/AIDS, STI, and TB services in California. Results suggest that accessing health services is related to education, acculturation, and attending health fairs as well as to positive disease prevention outcomes, such as increased access to condoms and better HIV risk knowledge. These results support continuing and expanding, perhaps in bi-national fashion, health educational and outreach efforts with Mexico-California migrants.

HIV and Latinos: Social and Behavioral Risk Factors

Prevalence and Correlates of Chlamydia trachomatis Infection among Mexican Migrants in Fresno County

Presenter: David Luchini, Department of Community Health, Fresno

Collaborator: Shahla Rahmani

Principal Investigator: David Luchini

UARP Award Number: CM02-FCDCH-803

Background: For five consecutive years (1999-2003) the Chlamydia trachomatis (CT) infection rate in Fresno County continued to be the highest in the State of California. In 2004, 4867 cases were reported to the Department of Community Health. This case count corresponds to a rate of 560 cases per 100,000 population and almost doubles the State average rate of 334. The highest reported cases were among 15-24 year olds, and the rate for females was three times higher than males, which reflects differential access to Chlamydia testing by females versus males. In 2004, the Department of Community Health collaborated with partners to implement the California–Mexico Epidemiological Surveillance Pilot (CMESP) in Fresno County. The goal of the project was to assess HIV and STI prevalence, risk behaviors and associated factors among Mexican migrants, for which there is no documented CT rate data in Fresno County.

Methods: From March 2004 to November 2004, 340 Mexican migrants were recruited through targeted random sampling in rural and urban areas in Fresno County, California. Recruitment sites included dwelling, work, and leisure venues. A 35 minute survey instrument was used to assess the risk behaviors and urine samples were taken for STI testing.

Results: Of the 336 persons who were tested for CT, 6.0% (20/336) were positive for infection: 6.6% of males (N= 227), 3.8% of females (N=105), and 25% of transgenders (N=4) were positive. The CT infection rates analyzed by reported sexual partners in the past 12 months break down as follows: 8% among MSF (N=165), 2% among M no partners (N=41), 4% among FSM (N=83), 5% among F no partners (N=20), and 25% among transgenders with male partners (N= 4). Factors associated with CT infection among males were further analyzed. The CT rate was 10.8% among males 18-25 years of age (N=102), 6.3% among males 26-35 years, and 0% among those over 35 years. CT infection rates analyzed by sexual behavior in the past 12 months break down as follows: 13% among males who engaged in sex work (N=38), 6.8% among males with one to two partners (N=148), and 10.3% of people with three and more partners (N=39). The incidence rate among migrants with five years or less in California was 7.3 %(N=138) and 5.6% (N=89) for migrants with more than five years. Infection rates considered with respect to language-based acculturation were 5.9% among low acculturation (N=193) and 6.7% of Medium/ High (N=34) acculturation. Logistic regression analysis among males including all of the factors above resulted in age group being the only significant variable associated with an outcome of CT infection.

Conclusion: Our findings indicate that the rate of Chlamydia infection among Mexican migrants in this study was ten times higher than the surveillance rate among the general population in Fresno County, and the rate was higher among males than females. These findings suggest that there is a need for tailored prevention programs, active screening strategies, and better access to STI diagnosis and treatment services for this population.

HIV and Latinos: Social and Behavioral Risk Factors

Increasing HIV Testing through a Health-promotion Focus

Presenter: Frank Galvan, Charles R. Drew University of Medicine & Science

Principal Investigator: Frank Galvan

UARP Award Number: CR02-DREW-600

Background: Latinos are disproportionately impacted by HIV/AIDS. In addition, they have been found to be less likely to have their HIV seropositive status detected early compared to non-Latino Whites. Failure to get tested early for HIV results in a delay in accessing treatment services for those infected and also increases the possibility of others' being infected. Thus it is important to identify ways to increase early HIV testing among Latinos engaging in high-risk behaviors.

This study sought to examine whether "bundling" (combining) HIV testing with tests for other conditions would increase HIV testing among Latino men who frequent gay bars compared to when HIV testing is offered by itself. It also sought to examine if bundling HIV testing would result in more HIV-positive individuals being identified compared to when HIV testing is offered by itself.

Methods: 394 Latino men who frequent gay bars were recruited. HIV testing was conducted at three gay bars catering to Latino men who have sex with men. On one night, randomly selected individuals were offered only the HIV test (the HIV-only protocol). On a different night, randomly selected individuals were offered a number of tests (for HIV, syphilis, gonorrhea, chlamydia, alcohol problems, drug dependence and depression) (the "bundled tests" protocol). The protocols were offered on alternate weeks on matched nights (for example, on alternate Saturday nights). As each randomly chosen individual was approached by an outreach worker, the outreach worker documented that contact as "consented to participate," "refused" or "ineligible."

Results: 3,645 individuals were approached in the course of the study. Of these, 11% agreed to participate, 88% declined and 1% were ineligible. The bundled tests protocol had a rate of 10.2% of individuals' agreeing to test for HIV, in contrast to a rate of 8.9% for the HIV-only protocol. This result was non-significant. In addition, an HIV-positivity rate of 3.4% was obtained with the individuals in the bundled tests protocol in contrast to one of 5.1% with those in the HIV-only protocol. This result, too, was non-significant.

However, multivariate analysis looking at subpopulation differences found that the following Latino men were more likely to test for HIV when it was bundled with screenings for other conditions: men who had sex primarily with women, men who reported having one or more risk factors, and men who were interviewed in a bar in a suburb far away geographically from the areas typically identified as being gay (e.g., with large numbers of gay-owned businesses).

Conclusion: Despite the fact that overall no statistical differences were found between the two protocols with regard to the number of individuals who took the HIV test, some important subpopulation differences emerged. The results of this study suggest that HIV testing using a bundled tests protocol may be most effective among Latino men who frequent gay bars but have sex primarily with women, Latino men with other risk factors that could also be tested through a bundled test protocol, and Latino men who are clients of gay-oriented bars that are outside of the immediate geographical proximity of a larger established gay community.

Bundling HIV testing into a broader health promotion protocol could encourage HIV testing among people who perceive themselves to be at low risk for HIV but may in fact be at high risk and at risk for passing it on to others. Thus this study suggests that there may be benefits to offering the HIV test in a "bundled package" to different subpopulations of Latino men. Further research can help to identify other potential benefits and challenges of bundling the HIV test for use with other populations.

HIV and Latinos: Social and Behavioral Risk Factors

Business Environments against the Transmission of AIDS (BEAT AIDS)

Presenter: Liza Rovniak, San Diego State University Research Foundation

Collaborators: Melbourne Hovell, Carol Sipan, Elaine Blumberg, C. Richard Hofstetter, Ana Martinez-Donate, Marcia Batista

Principal Investigator: Liza Rovniak

UARP Award Number: ID04-SDSU-060

Background: More than 72,000 Californians are HIV-infected and an additional 125,173 Californians have been diagnosed with AIDS. Currently, most HIV prevention programs are based in health clinics and community centers and are not reaching substantial numbers of people at risk for HIV-infection. The current project explores the feasibility of bringing HIV prevention into business settings. At-risk people visit businesses in their community every day, but little research has explored strategies to engage business owners in AIDS prevention.

Methods: Approximately 40 businesses located in Hillcrest, San Diego, will be engaged in distributing condoms and HIV-testing flyers to their customers and employees. Condoms will be wrapped in cute packages with humorous slogans. We are conducting a process evaluation of the feasibility of this approach. Process measures will include number of condoms distributed, types of condom packaging and slogans preferred by different businesses, business participation rate, business owner satisfaction with the program, and customer reactions.

Results: Preliminary pilot testing with 8 randomly selected business owners in Hillcrest, San Diego indicated that about 50 percent would be willing to display condoms or other AIDS prevention materials if given these materials free of charge. Process measures being collected from business owners and customers will provide further data on the viability of engaging business owners in AIDS-prevention initiatives.

Conclusion: This is the first study to address the extent to which businesses of different types are willing to display free condoms and other AIDS prevention materials at their business sites, and to determine satisfaction with this type of program. Lessons learned will guide future HIV-prevention interventions with at-risk groups.

HIV and Communication: Dissemination, Technology Transfer and Translation

Translating Street Smart in Partnership with Its Consumers and End-users: Technology Exchange as an Iterative Process

Presenter: George Ayala, AIDS Project Los Angeles

Collaborators: Rosemary Veniegas, Matt Mutchler, Monica Nuno, Oscar De La O, Richard Zaldivar, Emily Elman, Daniel Pierce

Principal Investigator: George Ayala

UARP Award Number: TR02-LA-500

Background: There remains a narrow range of available, well-evaluated, and effective HIV prevention interventions that target men who have sex with men (MSM), and even fewer for Latino MSM. The adaptation of already available evidence-based interventions and their successful adoption hold promise in expanding the range of available interventions that are more tailored to the specific needs of a target population. However, to date relatively few research studies have been conducted on the process of translating evidence-based interventions for implementation in community-based organizations (CBOs) that serve MSM, particularly MSM of color.

Methods: Investigators conducted a three-year, multi-method study that aimed to document the processes of adaptation and adoption of "Street Smart," an intervention originally designed for homeless and runaway youth. The intervention was adapted for use with young Latino MSM and delivered by staff from four Latino-run CBOs that vary along a specified set of organizational domains (size, age, staff characteristics, scope of prevention portfolios, and ideological commitment to both the target population and HIV prevention). To identify key factors likely to influence the successful adoption of Street Smart, an embedded case study was conducted over an 18-month period following a nine-month planning and training phase. Multiple data collection strategies were employed (focus groups with young Latino gay men who were the clients of the adopting agencies – 7 groups (n=42), semi-structured interviews with staff informants (n=10), document analyses, and brief client satisfaction surveys (n=35) administered during and immediately following the intervention) to generate detailed descriptions of the technology exchange process. In addition, quality assurance of fidelity was conducted through independent observation of video taped sessions of the intervention being implemented to assess fidelity to core elements of the adapted intervention. Client behavioral risk data were collected prior to and immediately following the intervention as a strategy for assessing the adapted intervention's potential efficacy (n=54 at baseline, n=33 at post-intervention). The investigative team kept in-depth field notes in the form of a log to record inter-organizational communications, staff changes within adopting agencies over time, and process issues related to the delivery of the adapted intervention. Organizational documents (i.e., agency mission statements) were collected and analyzed.

Results: Interviews with consumers and staff at adopting agencies suggested important ways to adapt the intervention in terms of content and pedagogy. Our findings support previously discovered links between organizational factors and successful adoption of HIV prevention interventions. Our data also suggest new organizational factors important to consider and raise new questions about the technology exchange process as currently conceptualized.

Conclusion: Our findings raise important questions for contemporary public health policy and HIV prevention practice that has begun to systematically endorse narrow guidelines for the adaptation and adoption of evidence-based interventions. An iterative model of adaptation that seeks ongoing consumer (members of the target population) and staff (end-users of the intervention) input may be a more appropriate approach.

HIV and Communication: Dissemination, Technology Transfer and Translation

A Web-Based Structural HIV-Prevention Intervention in MSM Networks

Presenter: Deb Levine, Internet Sexuality Information Services, Incorporated

Collaborators: Alberto Curotto, Greg Rebchook

Principal Investigator: Deb Levine

UARP Award Number: IP04-ISIS-603

Background: The Internet allows men who have sex with men (MSM) to find new sex partners and practice sexual behaviors that may be risky for HIV. Outbreaks of sexually transmitted diseases and cases of HIV infections have been traced to sexual networks formed in specific chatrooms. In response, community-based organizations and public health departments have begun using the Internet in their HIV-prevention programs. This study will develop a website to test new ways to prevent HIV transmission among California MSM using the Internet.

Methods: We are currently in the formative research phase of this project, and we have conducted four focus groups of MSM who meet sexual partners online (n=38). We recruited men from Internet venues to attend the in-person groups in Los Angeles, San Jose, Orange County, and Fresno. We asked participants for their reactions to our preliminary ideas about the website's features, their thoughts about how the Internet can be used to encourage MSM to reduce risk behaviors, ideas about using the Internet to promote community health and resilience, suggestions for the website design, marketing, and branding. We took detailed notes of each focus group and are currently conducting a thematic analysis of the qualitative data. Based on our preliminary findings (below), we are now working with web designers and programmers to develop the website which we will later beta-test, revise, launch, and evaluate.

Results: Several themes emerged that were directly relevant to the design of the website. First, the men in the groups expressed that they wanted opportunities to socialize and communicate with each other about common interests and concerns, including sexual health, in a more in-depth and genuine fashion than occurs in most online venues. Some men felt that the Internet can potentially reduce feelings of isolation, and many expressed the need for websites that, besides opportunities for hook-ups, would offer more ways to interact in fun and interesting ways, including in the offline world. Some of the desired features include events calendars, links to local resources, opportunities to get sexual health information, and instruction on writing safe and effective online profiles. In general, men wanted a site that was cleanly designed, functional, easy to search, non-judgmental, practical, and not preachy. The group discussions often turned to participants' likes and dislikes about current online venues. While no consensus emerged about the particular sites, it was clear that the men wanted a forum where they could share their online experiences and opinions of different websites with each other.

Conclusions: The groups endorsed a site with the following features: (1) Blogs: 3 featured bloggers (with at least one openly HIV+ blogger) will talk about issues of importance to MSM in California, including HIV prevention-related subjects, online cruising tips, resources, and community events. This will include a user comment area where men can have in-depth discussion about the issues presented; (2) Site reviews: An area containing an overview of hook-up sites and space for user reviews and ratings (similar to Cnet.com or Amazon.com); (3) Little Black Book: Application where users can organize information about their sexual encounters and networks, online buddies, HIV/STD testing history, and a personalized calendar to remind them when to get tested based on their actual risk behavior. This will be available online in a password-protected area, as well as downloadable to their own computers and/or handheld devices; (4) Safer sex content: Articles written by health professionals with resource and referral links, and the possibility for users to ask specific questions.

HIV and Communication: Dissemination, Technology Transfer and Translation

HIV Technology Transfer in Los Angeles: Preliminary Data on Evidence-Based Interventions Delivery

Presenter: Rosemary Veniegas, University of California, Los Angeles

Collaborators: Ricardo Rosales, Uyen H. Kao

Principal Investigator: Rosemary Veniegas

UARP Award Number: ID05-LA-024

Background: Community-based organizations (CBOs) have been strongly encouraged to implement evidence-based HIV prevention interventions for their clients. Many CBOs have begun conducting these interventions with limited guidance on how to adapt and tailor them for new target populations. CBOs' perspectives on the factors predicting successful or challenged implementation are of great interest to community planners, public health officials, intervention developers and funders alike. The Technology Transfer Model (TTM, Kraft et al., 2000) describes a three-phase (Pre-Implementation, Implementation, Maintenance and Evolution) process leading to successful replication of evidence-based interventions. This study will identify specific strategies that Los Angeles CBOs have employed to ensure success and overcome barriers during each of the TTM phases. Specific technology transfer training and technical assistance needs will also be identified at each TTM phase.

Methods: Eligible participants will complete close-ended background surveys regarding their organization's characteristics and will be interviewed for up to 90 minutes. Staff will be interviewed twice over the course of the study. The first wave of interviews will ask about pre-implementation and implementation phases of technology transfer. The second wave of interviews, scheduled when many of the programs will finish at least one cycle of implementation, will ask about maintenance and evolution (e.g., ensuring the presence of staff that can continue to implement the intervention). Up to 36 CBO staff with experience carrying out evidence-based interventions will be recruited for this study.

Results or Expected Results: CBO staff recruitment has begun. A total of 23 CBOs in Los Angeles were identified that were implementing 36 programs based on 12 distinct evidence-based interventions. A total of 37 CBO staff were identified as potential participants and invited to participate in the study. Seventy-one percent of these staff were working on interventions targeting men who have sex with men (MSM), 32% were targeting individuals who were HIV-positive, 24% were targeting men who have sex with men and women (MSMW), 22% were targeting women at sexual risk (WSR), 8% were targeting MSM who inject drugs, and 8% were targeting transgenders at sexual risk or who were injection drug users. Heterosexual male injection drug users and female injection drug users were each targeted by 5% of CBO staff. Information on which behavioral risk groups were being targeted was missing for three (8%) of the 37 staff. Los Angeles County has eight Service Planning Areas (SPAs) corresponding with different regions. Thirty-two percent of staff were conducting their interventions in SPA 2 (San Fernando), 27% percent of staff were conducting their interventions in SPA 8 (South Bay), 24% in SPA 3 (San Gabriel), 24% in SPA 6 (South), 16% in SPA 7 (East), 14% in SPA 4 (Metro), 8% in SPA 5 (West), and 5% in SPA 1 (Antelope Valley). Information regarding which SPAs were being targeted was missing for eight (22%) of the 37 staff.

Conclusion: The majority of staff were targeting MSM, followed by HIV-positive individuals, MSMW and WSR. More than a quarter of staff were conducting their evidence-based interventions in the San Fernando Valley and South Bay regions. Notably, the concentration of evidence-based interventions was higher in areas outside the Metropolitan region, which has the highest non-AIDS HIV percentage in the County. In light of these preliminary data, technology transfer resources may need to be dispersed across the entire span of Los Angeles County rather than focused in one or two areas.

HIV and Communication: Dissemination, Technology Transfer and Translation

Evaluation Systems for HIV Prevention: Development, Structure, and Training

Presenter: Shanna R. Livermore, California Office of AIDS

Collaborators: David Webb, Kevin Sitter, Valorie Eckert, Christine Dahlgren, Steven Truax

Background: There are many benefits of process evaluation including monitoring program implementation, fidelity to core elements, identification of target populations being reached, assuring appropriate geographic distribution of services, training needs, planning, policy making, and resource allocation. The State of California currently has three main process evaluation systems.

Methods: The *HIV Counseling Information System (Version 6.0)*, called HIV6, is desktop software developed in collaboration with local health departments (LHDs) and community based organizations (CBOs) for recording, processing, and reporting administrative and behavioral data about clients who have accessed counseling and testing services provided at state funded testing sites California. The program's goals at the local level are to provide LHDs and CBOs with useful information for program development and evaluation and to make reporting and accounting as easy and accurate as possible.

Counseling and testing information is collected on the HIV Counseling Information Form (CIFs) for all services provided. It is entered into the HIV6 system so the data can be analyzed and to generate invoices for reimbursement. Data is forwarded for processing and payment via state data disks or email.

The *HIV Information Processing System* is desktop software used by the state to process data generated by HIV6 and keep training records for HIV counselors. HIPS transforms the raw data from HIV6 into a SAS data set and tracks inventory such as CIFs and lab slips. HIPS also allows comparisons between counselors trained and the services they provide. This system has been recognized by the Center for Disease Control and Prevention as a national model for program evaluation and quality assurance.

The California State Office of AIDS (OA) designed its newest data collection system for HIV prevention providers called *Evaluating Local Interventions (ELI)* for all E&P activities. The goal of the ELI system is for California's HIV prevention providers to be able to systematically collect and access information critical to effectively prevent HIV infection and evaluate their programs. The collaborative process began by conducting needs assessments across the State with the University of California AIDS Research Program (UARP) to define core measures that target program implementation and risk behavior. Data collection forms for various types of encounters were developed in conjunction with the system and are divided into intervention types: individual, group, outreach, prevention case management, and health communication and social marketing. Providers collect information using these forms and then enter data into the ELI system via the Internet. All three systems continue to evolve based on provider feedback.

Results: We will discuss the role of process monitoring in intervention development as well as the opportunity for research projects to utilize the OA process monitoring systems.

Conclusion: Participants of this session will be able to describe the process of development, structure and training for data evaluation systems in California and understand the basic benefits of process evaluation

HIV and Communication: Dissemination, Technology Transfer and Translation

HIV Risk and Identity in African American Men Who Have Sex with Men in California

Presenter: David S. Webb, Department of Health Services, California Office of AIDS

Collaborators: Valorie Eckert, Roy McCandless, Steven Truax

Background: HIV/AIDS has had a disproportionate impact on gay/men who have sex with men (MSM) racial/ethnic minorities, especially African American and Latinos. This study examines demographic, behavioral, and health history variables associated with HIV in a large cohort of African American gay/ MSM accessing publicly funded HIV sites in California.

Methods: Counseling and testing program data from the California HIV Counseling Information System (versions 5 and 6) were used for this analysis. Male clients who reported a male sex partner with valid tests results who had not tested positive previously from January 1, 2001 through August 31, 2005 (N = 151,527) were selected from these data.

Results: African American gay/MSM in this sample were twice as likely to test HIV positive (OR=1.94: 95% CI 1.80, 2.08) with an overall seropositivity rate of 7.3%. Compared to other MSM, African American MSM were more likely to be referred to testing through outreach, test in mobile vans and outreach venues, and fail to return for their test results. African American gay/MSM reported much higher rates of crack use and higher rates of self reported sexually transmitted diseases. In addition, these men identified as straight or bisexual and reported having sex with women at higher rates with higher rates of discordance between their sexual behavior and sexual orientation.

Conclusion: African American gay/MSM in this analysis demonstrated substantial risk for transmission of HIV infection. The differing sexual orientation and sexual behaviors indicate diverse subgroups within the African American MSM population. Prevention programs in gay identified venues and directed to gay identified men may not reach a large number of these men. Developing culturally specific HIV prevention programs that target these men are needed to increase rates of HIV testing.

HIV and Communication: Dissemination, Technology Transfer and Translation

Developing Additional Dissemination Technology

Presenter: Kevin Sitter, Department of Health Services, California Office of AIDS

Collaborators: Steve Truax, Shanna R. Livermore, Judith Fitzpatrick

Background: The Office of AIDS (OA) HIV Prevention Research and Evaluation Section reserve some of its funding for collaborative research and dissemination. It is currently developing a web-based system that will allow providers to review evidenced-based interventions that they can use to address HIV prevention with their target populations. This system will increase the ability for researched programs to be disseminated easier and link providers with others conducting the intervention, as well as with the original research team.

Methods: With a growing body of OA sponsored collaborative research projects being completed, mechanisms for dissemination are needed. As an option to published modules, a web-based system will place intervention information on-line, and link it to a search engine that will allow users to seek interventions appropriate for their target population. This will increase access and assist local health departments implement research-based interventions

Results: We will discuss the design of the website, including the search engine tool and means to get research on-line. A discussion of options to disseminate research will help identify elements that are useful to prepare research for dissemination and translation.

Conclusion: This new web-based system will provide a mechanism to disseminate research efficiently and is intended to increase the application of research-based interventions at the community level.

HIV and Men: Social and Behavioral Risk Factors

Bathhouse-based Voluntary Counseling and Testing Is Feasible and Shows Preliminary Evidence of Efficacy

Presenter: David M. Huebner, University of California, San Francisco

Collaborators: Diane Binson, William J. Woods, Samantha E. Dilworth, Torsten B. Neilands, LeRoy Blea, Olga Grinstead

Principal Investigator: Diane Binson

UARP Award Number: CR03-SF-520

Background: The goal of this study was to provide evidence for the feasibility and effectiveness of conducting voluntary counseling and testing for HIV in a bathhouse setting.

Methods: Four hundred ninety-two men participated in bathhouse-based VCT offered at a single venue over a 13-month period. A convenience sample of 133 of these testers was assessed at two points: immediately prior to and three months after testing.

Results: Thirty-eight percent of men in the sample reported unprotected anal intercourse with one of their two most recent partners in the 3 months prior to testing, and 48% of those men had not otherwise tested for HIV in the previous 12 months. Results showed that in the months following VCT, men decreased their frequency of engaging in sex while drunk or high, and were more likely to communicate about HIV and condom use with their sexual partners. Although the overall number of unprotected anal sex acts did not change from baseline to followup, the proportion of the sample reporting unprotected sex at followup was significantly smaller than the proportion reporting risk at baseline.

Conclusion: Bathhouse-based VCT appears to be a feasible approach for reaching significant numbers of men at risk for HIV and shows preliminary evidence of effectiveness in changing HIV-related risk and precautionary behaviors.

HIV and Men: Social and Behavioral Risk Factors

The ES-HIM Intervention for Seropositive African American and Latino MSM with CSA Histories

Presenter: John K. Williams, University of California, Los Angeles

Collaborators: Gail E. Wyatt, Judith Resell, Hema C. Ramamurthi

Principal Investigator: John K. Williams

UARP Award Number: CC02-LA-001

Background: For the past 25 years, the HIV epidemic has disproportionately affected men who have sex with men (MSM) in the United States. For African American and Latino MSM, the HIV incidence and prevalence has been especially pronounced. Further, African American and Latino MSM with histories of child sexual abuse (CSA), an important predictor of high-risk sexual behaviors, and who are already infected with HIV, are particularly vulnerable populations for high-risk sexual behaviors and negative psychological sequelae such as depression. As HIV continues to spread among this population, it is important to develop interventions to reduce HIV transmission.

Methods: The Enhanced Sexual Health Intervention for Men (ES-HIM), guided by cognitive-behavioral approaches, the Social Learning Model, and the efficacious Women's Health Project, was designed for HIV positive gay and non-gay identifying African American and Latino MSM with histories of CSA. A randomized controlled trial examining depressive symptoms, number of sex partners, and condom use intentions was compared among intervention and control groups with pre-post testing from 1999-2005. The trial compared two 6-week, 120-minute sessions, a sexual risk reduction intervention (ES-HIM) and a comparison standard health promotion condition (HP), implemented by ethnically matched male health educators.

Results: The study included 120 African American and Latino MSM with histories of CSA, ES-HIM (n=60) versus HP (n=60). The sample was marginalized with no significant differences on mean age (ES-HIM = 44.1 years vs. HP = 43.0 years) or on mean years of education (ES-HIM = 13.0 vs. HP = 13.6 years), and both groups were predominantly unemployed (ES-HIM = 88.9% vs. HP = 86.7%) with mean monthly incomes of $676. Using the CES-D, the ES-HIM Intervention was efficacious in significantly decreasing depressive symptoms ($P<0.01$) at post testing. While both groups decreased their mean number of partners in the previous 30 days, only the Intervention had significant pre-post change in number of partners from 3.02 to 2.03 ($p<0.001$). Regarding condom use intention, only the ES-HIM Intervention showed significant pre-post change ($p<0.04$).

Conclusion: This unique gender and culturally specific intervention focused on perceived male roles and culture, the effects of coercion in relationships, and the importance of condom use in personal protection and protection of partners. Emphasis on these topics may have contributed to it being efficacious at decreasing depressive symptoms and number of sex partners, while increasing condom use intentions among these African American and Latino MSM who commonly lack such discussions. Future research must assess if these changes are sustained at 3-and 6-month post.

HIV and Men: Social and Behavioral Risk Factors

Outcome Findings from an Enhanced HIV Prevention Case Management Intervention for High-risk Transgender Women

Presenter: Cathy Reback, Friends Research Institute, Inc.

Collaborator: Mely D. Silverio

Principal Investigator: Cathy Reback

UARP Award Number: CR03-FRII-522

Background: Male-to-female (MTF) transgender women are at extreme risk of HIV infection due to several socio-cultural conditions (e.g., low income, high unemployment, lower levels of education, and unstable housing). Other factors specific to their transgender identity (e.g., hormone misuse and sex work resulting from lack of viable employment) also increase their HIV risk.

Methods: From February through November 2004, 60 high-risk transgender women enrolled in an enhanced prevention case management (PCM) intervention to receive a maximum of 10 PCM sessions. A baseline and six-month follow-up evaluation was conducted with participants. The impact of the PCM intervention was measured by the following outcomes: (a) reducing sex work by facilitating legitimate employment; (b) lowering HIV injection risks by helping transgender women to obtain legal and monitored hormones; (c) reducing substance abuse by helping transgender women with the decision to enter treatment; and (d) reducing homelessness by helping transgender women to obtain stable, affordable housing.

Results: Participants ranged in age from 20 to 64 years, with an average age of 38.3 years (SD=10.1). Predominantly non-white, the participants were 30% Hispanic/Latina, 28% Caucasian/white, 22% African Americans/black, 12% Native Americans/American Indians, and 8% other. Sixty-three percent identified as transgender, while none have undergone genital reconstructive surgery, 7% identified as transsexual and 28% identified as female. Half (50%) identified as heterosexual, 23% as gay, 18% as bisexual, 5% as lesbian, and 4% other. The participants completed an average of 11.5 (S.D.=2.8) years of education. HIV seroprevalence was 28%. Forty-five participants (75%) completed all 10 PCM sessions with a mean of 8.72 PCM sessions completed per participant (S.D.=2.58). Six-month follow-up evaluations were conducted with 97% (n=58) of the participants. From baseline to six-month follow-up evaluations, sex work as an income source decreased from 42% to 22% ($p<.05$); homelessness declined from 62% to 41% ($p<.05$); and the estimated index that measures the overall level of psychological distress lowered from 1.02 to 0.82 ($p.<05$). Moderate changes were found in the number of participants who reported legal employment, which increased from 18% to 22%; General Assistance/General Relief as a source of income declined from 37% to 29%; and hormone misuse decreased from 57% to 41%. Similarly, overall alcohol and substance use in the previous six months declined from 72% to 65% and injection drug also declined from 17% to 12%.

Conclusion: Findings from this study can inform policymakers on the level of services that produce maximal behavioral change in this vulnerable group of individuals at extremely high risk for HIV acquisition or transmission.

HIV and Men: Social and Behavioral Risk Factors

Correlates of Unsafe Sex among Adult HIV Positive Heterosexual Men

Presenter: Joel Milam, University of Southern California

Collaborators: Jean Richardson, Lilia Espinoza, Sue Stoyanoff

Principal Investigator: Joel Milam

UARP Award Number: ID04-USC-042

Background: Behavioral interventions promoting safer sex among people living with HIV may need to be tailored to the sexual behaviors and relationships of individual patients to be most effective. This study examines correlates of unprotected sex among specific subgroups of people living with HIV to determine whether easily assessed characteristics can be used as a basis for tailoring safer sex counseling in the clinic setting. Preliminary results are available for a subgroup of male heterosexual patients.

Methods: 121 adult HIV-positive heterosexual men who were attending one of six clinics in California and were sexually active with one partner in the prior 3 months were included. Potential correlates of self-reported unprotected oral (receptive) and vaginal sex included participant demographics (e.g., age, ethnicity), disease status (CD4 counts, viral load, years since diagnosis), safer sex beliefs (e.g., condom attitudes, safer sex self-efficacy), substance use, psychological characteristics (depressive symptoms, dispositional optimism and pessimism), and sex partner characteristics (main/casual partner, status, and duration of relationship). A series of logistic regression analyses were used to determine significant relationships.

Results: Correlates of reported levels of unsafe oral (24%) and vaginal (21%) sex were not associated with the type of relationship (main or casual) or perceived HIV infection status of the partner (positive, negative, or unknown). Preliminary results indicate that unsafe oral sex was positively associated with age and CD4 counts and inversely associated with optimism and positive condom attitudes (all p's$<.05$). Unsafe vaginal sex was inversely associated with positive condom attitudes.

Conclusion: Prevention efforts among sexually active adult heterosexual men living with HIV may benefit from focusing on improving attitudes towards condom use regardless of partner relationship status.

HIV and Men: Social and Behavioral Risk Factors

Asymptomatic Sexually Transmitted Infections (STIs) Are Common in HIV-Infected Men Who Have Sex with Men (MSM)

Presenter: Gunter Rieg, LA Biomedical Research Institute at Harbor-UCLA Medical Center

Collaborators: Loren Miller, Mario Guerrero, C. Aquino, Mallory D. Witt, Eric S. Daar

Principal Investigator: Gunter Rieg

UARP Award Number: ID03-REI-040

Background: STIs are common among MSM and associated with significant morbidity and increased HIV transmission. The CDC recommends annual screening for asymptomatic STIs among MSM, and more frequent screening for high risk patients. However, the utility of this approach has not been well addressed. Additionally, the added value of screening of rectal and pharyngeal sites for asymptomatic STIs is poorly defined.

Methods: We enrolled asymptomatic HIV-infected MSM, regardless of STI risk, from two urban clinics. Subjects completed a survey of STI risk factors and were prospectively screened for asymptomatic STIs at enrollment and after 6 months. STI screening was comprised of: 1) RPR/FTA testing, 2) Nucleic acid amplification testing (NAAT) for chlamydia (CT) and gonorrhea (GC) at urethral (urine), pharyngeal and rectal sites and 3) Pharyngeal and rectal GC cultures.

Results: Among 212 subjects, 28 (13%) tested positive for 35 STIs at baseline. GC and CT were found in 13 (6%) of subjects each, 18 of which were positive at rectum and/or pharynx and 5 in urine. RPR was newly positive in 5 (2%) subjects. Four subjects had >2 sites infected and both GC and CT was found in 2 subjects. At the follow up visit (n=179) 14 (8%) had a new STI, 10 of whom did not have an STI at baseline and 4 who were re-infected after treatment. GC and CT was identified in 6 (3%) each, 10 of which from pharynx and/or rectum and 1 in urine. RPR was newly positive in 4 (2%) subjects. Three subjects had >2 sites infected and multiple STIs (CT + GC and CT + newly positive RPR each) was found in 2 subjects. Among subjects with 0, 1, or >2 partners 6 months prior to enrollment 9% (6/70), 15% (8/52), and 31% (25/80) had an asymptomatic STI during the study period, respectively (p=0.0007). Risk factors for STI during the study included being sexual active (RR=2.18, p=0.008) and >2 sexual partners (RR=2.78, p=0.0006,referent group: no sexual partners) in the 6 months prior to the study. We found no association between anonymous sex, sex while using drugs or sildenafil with having an asymptomatic STI.

Conclusion: We found a high prevalence of GC and CT infection among asymptomatic HIV-infected MSM, especially at rectal and pharyngeal sites. Follow up screening demonstrated a high rate of incident infections at mucosal sites and supports the potential role of more frequent screening of mucosal sites in sexually active MSM regardless of self-reported STI risk.

HIV and Men: Social and Behavioral Risk Factors

Sexual Risk Behavior Chain Analysis in Persistently High-Risk Gay Men

Presenter: David Martin, LA Biomedical Research Institute at Harbor-UCLA Medical Center

Collaborators: Robert Chernoff, Michael Buitron

Principal Investigator: David Martin

UARP Award Number: ID04-REI-038

Background: Despite many years of HIV prevention and education efforts, certain gay men continue to engage in persistent high-risk sexual behavior. Certain factors such as childhood sexual abuse have been associated with persistently high-risk sexual behavior, but little is known concern the behavioral topography of high-risk behavior among this group, and existing interventions do not address potential antecedents to the risky behavior. Linehan's (1993) depiction of Borderline Personality Disorder and Quadland & Shattls' (1987) depiction of persistently promiscuous sexual behavior both suggest that high-risk sex may serve to regulate negative emotions. In both models, persistent self-destructive acts are culminations of sequences or chains of events that start with negative affect and that regulate the negative emotional state.

Methods: We began this study with a working hypothesis that persistent high-risk behavior may be the end of a chain of events, usually beginning with a personally invalidating event followed by negative affect and a sequence of behaviors intended to improve mood, reduce anxiety, and lead to environmental validation. To begin testing this hypothesis, we interviewed 58 gay-identified men who also identified themselves as engaging in persistently high risk, asking them to describe the most recent incident of unprotected anal intercourse and the events and behaviors preceding, using "chain analysis" (Linehan, 1993) techniques. All interviews were audiotaped and transcribed, and content analysis conducted to identify themes, using grounded theory. Thematic content was then coded and tallied to arrive at frequencies with which individual themes emerged.

Results: All participants were self identified gay men. Participants ranged in age from 18 to 65, with a median age of 38. Ethnicity: 59% White, 24% Latino, 10% African American, 7% Other. Serostatus: 62% identified as HIV+, 36% identified as HIV-, one participant didn't know his status. HIV+ participants had a mean CD4 count of 546. Median age at first sex with a man was 14. Drugs: 52% used methamphetamine, 29% used club drugs, 14% used barbituates, 9% used cocaine, 9% used crack. 48% used Viagra. The mean number of sex partners in the past 90 days was 24.5, 82% were unknown to the participants at the time of the sexual encounter. Condoms were NOT used 79% of the time. As expected, participants' accounts of the behavioral chains were frequently characterized by early non-validating events, depression, loneliness, or boredom, with the sexual encounters used to reduce the negative affect. Participants frequently described the sexual encounters as planned spontaneity (e.g., "I wasn't looking for anything to happen," "if it [sex] happens, it happens"), but these encounters typically happened in sex venues. Risk reduction was viewed as interfering with enjoyment, and HIV+ participants identified their serostatus as a reason for not being concerned about infection.

Conclusion: Current approaches to risk reduction are typically centered in public health models focused on health education, stages of change and skills building. Little attention has been paid to affective bases of behavior. These qualitative results suggest that, at least for a subset of individuals, interventions may need to address the emotions surrounding the high-risk behaviors and their antecedents, and focus efforts on earlier intervention in the chain of events leading to the risky behavior.

HIV and Men: Social and Behavioral Risk Factors

Migration and HIV Risk among MSM

Presenter: Joseph Catania, University of California, San Francisco

Collaborator: Lance Pollack

Principal Investigator: Joseph Catania

UARP Award Number: ID04-SF-008

Background and Methods: The HIV epidemic among men who have sex with men (MSM) is of substantial concern. Recent findings indicate HIV prevalence among MSM in San Francisco has increased from 20% in 1996 to approximately 28% in 2003. The proposed work examines unexplored population level processes influencing HIV risk behaviors and HIV distributions among MSM (sexual mixing, demographic changes, migration). In this phase of the work, we investigated the relationship between indices of MSM migration and HIV risk and prevention practices in survey probability sampled based research conducted at the City (Urban Men's Health Study III, 2002-03, N = 850) and State levels [California Health Interview Survey-MSM Survey, 2002-03; N = 395).

Results: Approximately 75% of adult MSM residents of California migrated into the state at some earlier point in their adult life. In San Francisco, approximately 80% of MSM are in-migrants from other locales. Preliminary findings at the state level indicate that more recent MSM in-migrants have higher levels of risk behavior, greater use of sex venues, and are less likely to have been tested for HIV; the age of in-migration, the number of moves made since age 18, and the number of MSM-urban centers lived in are related to HIV risk indices. In San Francisco (SF), MSM not born in SF are more likely to attend sex venues, have more sex partners, greater HIV risk, and greater sexual mixing. In addition, migrants from outside the US vs. US residents had higher levels of risk behavior, use of sex venues, less HIV testing, more sex partners, and less condom use. Recent in-migrants to SF had higher levels of risk behavior, less condom use and sero-sorting, more use of sex venues, more sex partners, and great sexual mixing than older in-migrants.

Conclusion: The findings suggest that new migrants/in-migrants to California and large urban MSM communities, are at greater risk for HIV infection; while native born MSM are at lower risk. This suggests that community norms for safe sex are strongest for native born MSM and weakest for in-migrants; implications for HIV prevention will be discussed.

HIV and Youth: Social and Behavioral Risk Factors

Sexual Health Communication: Young Gay Men and Their Friends

Presenter: Matt Mutchler, AIDS Project Los Angeles

Collaborators: Mark Schuster, Emily Elman

Principal Investigator: Matt Mutchler

UARP Award Number: ID05-APLA-036

Background: It is crucial to prevent the further transmission of HIV infection among young gay men (YGM). One way to reduce behaviors that may transmit HIV among YGM is to foster strong sexual communication skills with their sexual partners. However, we do not know enough about the content and quality of sexual health communication between YGM and their friends. Friendships with other YGM and heterosexual females are particularly important sources of support for YGM. Thus, our study explores how sexual health communication between YGM and their best gay male and best heterosexual female friends may be related to healthier sexual lives among the YGM.

Methods: This study is guided by the theories of planned behavior and sexual scripts. We will purposefully sample gay male participants with best gay male or best heterosexual female friends and will conduct in-depth, semi-structured interviews with 24 dyads (12 gay male/best gay male friend dyads and 12 gay male/best heterosexual female dyads). Our sampling matrix consists of 6 Caucasian, 12 (4 English-speaking and 4 Spanish-speaking) Latino, and 6 African American dyads matched by race/ethnicity. Participants for the study will be recruited at gay venues and invited to participate in a series of interviews: first as a dyad and second, individually. Participants will also complete a brief quantitative survey including items on friendship characteristics, sexual risk, and communication regarding sexual health issues.

Expected Results: The findings from this study will have important implications, not only for YGM in Los Angeles, but also among similar populations at risk for HIV infection in California and nationally. We expect to discover how young gay men talk about sexual health issues (or do not) differently with their gay male versus heterosexual female friends and by race/ethnicity.

Conclusion: We will use the findings to: learn about the topics of sexual health communication that YGM discuss with their best gay male and heterosexual female friends; form an HIV prevention intervention that targets friendship dyads; and assess receptivity to HIV intervention topics and formats among YGM and their best friends. Our presentation will highlight the significance of the study; the study design; measures; and plans for data collection, analyses, and dissemination.

HIV and Youth: Social and Behavioral Risk Factors

Outreach-based STI Testing Accesses a Higher-risk Group of Youth in Comparison to Clinic-based Testing

Presenter: Eiko Sugano, University of California, Berkeley

Collaborators: Dina Wilderson, Shelley Facente, Teri Dowling, Kate Scott, Jeffrey Klausner, Michael Baxter, Susan Obata, Benjamin Hickler, Colette Auerswald

Principal Investigator: Colette Auerswald

UARP Award Number: CR04-SF-502

Background: Homeless youth suffer from disproportionate rates of STIs and HIV. The highest risk youth are not found in clinics or shelters, but on the street. Urine-based methods for gonorrhea and chlamydia screening and rapid tests for HIV have made it possible to deliver STI/HIV screening services to youth in non-clinic settings. The Street START Project is a collaboration of non-profit, governmental, and academic partners to create a model program for street-based STI and HIV screening, treatment and linkage to care of homeless youth in San Francisco by Larkin Street Youth Services (LSYS) outreach staff.

Objectives: Our specific aims are: 1) To determine the acceptability and feasibility of outreach-based testing for chlamydia and gonorrhea and to dispense field-based therapy, and 2) To determine the acceptability and feasibility of outreach-based rapid HIV testing. We hypothesize that outreach-based testing will reach a high-risk population of youth, that outreach-based STI treatment will be feasible and acceptable, and that outreach-based HIV testing will reach a higher percentage of youth testing for the first time than clinic-based testing.

Methods: Phase 1: Qualitative assessment of the feasibility of outreach-based STI and HIV testing using focus groups and key informant interviews with street youth, outreach workers and providers. Phase 2: Implementation of joint LSYS-UCSF outreach-based STI testing. Phase 3: Implementation of joint LSYS-UCSF outreach-based STI and rapid HIV testing Phase 4: LSYS outreach-based STI and rapid HIV testing with UCSF consultation.

Results: Phases 1 and 2 are completed. Issues elicited during the focus groups and interviews included confidentiality in the outreach setting, the importance of recognizing neighborhood-specific youth needs, the impact of the study on outreach activities, and obstacles to obtaining necessary contact information for treatment of STI-positives and referral to care of youth testing preliminarily positive for HIV. Since May 2005, 86 street-based youth and a comparison group of 51 clinic-based youth have been recruited. Street-based youth were more likely to be male (74% vs. 45%, $p<.01$), White (64% vs. 41%, $p<.01$) and older (mean age 22.6 vs. 20.3, $p<.01$). Street-recruited youth were more likely to engage in HIV-risk behaviors, such as having a higher mean number of sex partners in the past 3 months (5.2 vs. 2.2, $p<.05$), not using a condom at prior vaginal sex (61% vs. 40%, $p<.05$), having had a sex partner who was an injection drug user (43% vs. 10%,$p<.01$) or who was perceived to be HIV-positive (30% vs. 10%,$p<.01$), ever engaging in survival sex (47% vs. 20%, $p<.01$), or ever injecting drugs (48% vs. 4%, $p<.01$). No significant differences were found in prior STI testing history between the two groups (96% vs. 89%, $p=.11$). Street-based youth were more likely to report having ever been tested for Hepatitis C and HIV (78% vs. 46% and 90% vs. 67%, respectively; both $p<.01$). If tested, street-based youth were more likely to report testing positive for Hep C (32% vs. 0%, $p<.01$). There was no statistical difference in STI rates in the two groups. All STI-infected street-based youth have been treated.

Preliminary Conclusions: Our preliminary results suggest that street-based STI testing is accessing a higher-risk subgroup of youth, but, unexpectedly, a group with higher rates of prior HIV testing relative to clinic-based youth. The implementation of outreach-based HIV testing will enable us to assess whether street-based rapid HIV testing attracts street-based youth who would be testing for the first time.

HIV and Youth: Social and Behavioral Risk Factors

An Ethnographic Study of the Production of Scientific Sexual and Drug-Use Knowledge of and about Homeless, Sexual Minority Young People in San Francisco

Presenter: Ben Peacock, University of California, Berkeley

Principal Investigator: Ben Peacock

UARP Award Number: D05-B-409

Background: In ongoing ethnographic research with homeless sexual minority young people (15-28), I am considering how their stigmatized and illegal behaviors are often concealed by not only themselves, but also by cultural and structural forces within the local gay and larger communities, local and national governments, and scientific practice itself. While identifying these forces (discursive, violent, political, legal, economic, symbolic, epistemological) I came to see the increasing importance of quantitative behavioral survey research since the advent of the AIDS Pandemic to enumerate—and thereby reveal—stigmatized and illegal behaviors. For example, when exploring my informants' participation in survival sex, explicit speech about it was constrained. But contrary to the theorizing of anthropologists on the inexpressibility of suffering and pain in language, I learned that it was not that the youth cannot not speak about their participation in survival sex, but rather that their speech and actions about it are not heard. My ability to label or translate such speech and actions came in part from the work of behavioral epidemiologists to assemble a variety of behaviors into the category of 'survival sex'. As a result I bore a kind of knowledge that many I observed lacked, a sexual knowledge generated from the analysis of data abstracted from others 'like' them in the past. While the quantitative social sciences have been critiqued for concealing structural oppression and the politics of research, the research on survival sex provides a counterexample of how they can also be used in attempts at the opposite. This led me to consider how numbers and enumeration can be used to reveal behavior which other cultural forces serve to conceal or elide, and thereby expanded my dissertation project to consider both the human researchers and researched humans in the production of sexual and drug-using knowledge.

Methods: The majority of my data is constituted by my detailed field notes and interviews with homeless (25) and scientific (15) informants. I have been accompanying my homeless youth and young adult informants in many activities of their daily lives and my scientific informants in their professional practices, including project planning, funding activities, instrument design, writing up and public presentation of findings, and recruitment, outreach and surveying of research subjects.

Results/Conclusion/Relevance: This project seeks to understand how epidemiological categories of sexual behavior and 'at-risk' populations are constituted; a critical feature of this analysis examines how behavioral epidemiologists and the humans they study interact to produce scientific sexual knowledge. This project therefore links an anthropology of science attentive to knowledge practices with an urban anthropology rooted in gay and lesbian/queer studies that foregrounds the subjectivity of stigmatized groups. To understand the relation between the lived experience and social worlds of persons and the scientific practices by and in which they are transformed into a sexual category of risk, I am examining the everyday practice both of behavioral epidemiologists and their human objects of study.

HIV and Youth: Social and Behavioral Risk Factors

HIV Intervention Project (HIP)

Presenter: Donnie Watson, Friends Research Institute, Inc.

Principal Investigator: Donnie Watson

UARP Award Number: ID04-FRII-073

This pilot project, entitled HIV Intervention Program (HIP), is submitted to the Universitywide AIDS Research Program in the Innovative, Developmental, Exploratory, Awards (IDEA) category. HIP is a pilot study that will assess the feasibility of adapting a research supported intervention (i.e., Street Smart) to an extremely high risk California population of predominantly Hispanic (60%) and African American (30%) juvenile offenders who, while detained in 24-hour male only residential youth correctional facilities, attend school on-site. The targeted youth exhibit a constellation of behaviors that include risky sexual behavior and substance use. Since they are detained in this secure setting for 4 months before returning to the community, HIP will address their sequelae of HIV risk behaviors by providing intensive after school interventions delivered by master's level clinicians. The specific aims of this project are to: 1 – adapt Street Smart for use with the target population by enlisting certified Street Smart Trainers to train the clinicians who will deliver the intervention; in addition to ensuring fidelity, this training will also include content review regarding cultural relevance for the target group of adolescents; 2 – conduct a randomized pilot to assess the preliminary short-term utility of the intervention in reducing HIV risk behaviors. The proposal is novel in that it will adapt an evidence-based HIV prevention program (i.e., Street Smart, Rotheram-Borus et al., 1991; 2003) for use with this understudied population in a unique setting. In order to accomplish this, the intervention will be delivered systematically over 2 months as part of their after school programming. HIV risk behaviors and associated risk behaviors will be examined as short-term outcome measures. Outcomes will be assessed at baseline and 6 months (i.e., 2 months after release). The outcomes for a total of 80 participants (i.e., 40 youth who receive the intervention Vs 40 participants in a comparison group) will be evaluated. We hypothesize that participants in the Street Smart will have better outcomes than those in the comparison group. The study results will inform the California community of researchers and providers about the feasibility of implementing Street Smart with the target population while providing pilot data for larger scale studies.

HIV and Youth: Social and Behavioral Risk Factors

Early Adolescent Response to Peer Videos Related to Sex

Presenter: Stephen Eyre, University of California, San Francisco

Principal Investigator: Stephen Eyre

UARP Award Number: ID02-SF-026

Background: The goal of this research is to learn how early adolescents respond to peer-authored videos conveying messages about sex. The long-term goal of this research is to develop improved sex education for this age group by using peer-authored videos to activate peer culture, making sex education more authentic.

Methods: Eighteen students at a low-income inner city middle school (9 female and 9 male, ages 13 to 14 years, mixed ethnicity) were shown a set of 4 peer-authored videos that conveyed messages about sex. At a later time, individual participants were re-shown one video that they had rated highly and were interviewed about that video. The 4 videos addressed topics of 1) gossip and relationship sabotage, 2) rape, and 3) and 4) discovery of pregnancy. Interviews were open-ended, allowing participants to move from discussion of the video to other topics of interest to him or her. A team of two researchers analyzed these interviews, independently identifying topics discussed at length in each interview. A category system was developed to reduce the number of topics and to determine the frequency of topics.

Results: In descending order of frequency, the 9 topic categories discussed by more than one participant were as follows: 1) Pregnancy-related (11), 2) Family (8), 3) Sexual choices (4), 4) Popularity (4), 5) Aspirations (4), 6) Fighting at school (3), 7) Gangs (2) 8) Fun (2), and 9) Drugs (2). Participants, both female and male, have well developed views about the choice between keeping, giving up for adoption, or aborting a child. Many participants adhere to their parent's advice, feel that their parents trust them, and could expect disapproval but also support were they to become sexually active or cause a pregnancy. Participants see having sex as a bad choice, something that should be deferred until after finishing school or until marriage. Participants locate themselves with reference to a popular group and believe that desire for popularity can bring unwanted change through "peer pressure." Participants express a desire to "be somebody." Pregnancy is seen as likely to stand in the way. Fighting is seen as "natural," interesting, and guaranteed to draw a crowd at school. It releases anger. Students at this school are surrounded by gangs in home neighborhoods and tell stories of gang violence. Fun is seen as a prerogative of middle school students and pregnancy is seen as likely to interfere with it. Cigarettes are the main "drug" participants have to deal with and it is seen as a weakness to start smoking.

Conclusions: The results of this study show that peer-authored videos that convey messages related to sex activate thinking about a range of topics only some of which are directly related to sex. The videos appear to evoke an early adolescent world, which includes family and gangs, both out of school topics, as well as popularity, fighting, and fun, topics more closely tied to school.

HIV and Youth: Social and Behavioral Risk Factors

A Parent-Adolescent Program to Promote Healthy Sexual Development and Reduce HIV Risk

Presenter: Mark Schuster, University of California, Los Angeles

Collaborators: Katherine Vestal, Paul Chung, Wen-Chih Yu

Principal Investigator: Mark Schuster

UARP Award Number: ID04-LA-062

Many U.S. adolescents engage in sexual activities that put them at risk for HIV, other STDs, and unintended pregnancies. At least half of new HIV infections are estimated to be among people younger than 25 years old. Some adolescent HIV prevention programs have shown effectiveness in reducing risk behaviors, but program effects often do not endure. Research suggests that parents can have a strong influence on their child's behaviors through parenting and communication behaviors that reduce risk. A promising yet relatively new approach for increasing the effectiveness and duration of such prevention programs is to incorporate parents along with their adolescent children. It can be a challenge, however, to recruit parents and children to participate in a program that deals with such a sensitive and potentially stigmatizing topic. Many parents avoid discussing sex with their children because they feel uninformed and inhibited, or fear that talking about sex will "encourage" sexual activity. There are also many other important health issues that concern parents, such as substance use and obesity. One approach that addresses these points is to provide HIV prevention education in the context of broader health issues. Incorporating additional topics may give a program wider appeal while reducing the stigma of participating in a program about sex and HIV. Our program helps parents and adolescents become more comfortable and skilled at communicating with each other about sensitive issues, with the goal of creating a fundamental change in the parent-adolescent relationship and a family environment that encourages adolescents to make healthy choices. Our Center previously designed a program to promote healthy adolescent sexual development and risk reduction by helping parents learn skills to communicate effectively with their adolescent children. We are now expanding this program to include adolescents along with their parents and to cover multiple topics. The adapted program will aim to help parents and adolescents learn more effective approaches for interacting with each other while simultaneously imparting knowledge related to sexual health and development, substance use, and obesity so that parents and adolescents will be able to communicate about these sensitive topics in an open and constructive manner. We are conducting formative research to guide and refine the adaptation of the program as well as a pilot study of the program with middle school students and their parents. The formative research consists of key informant interviews with school personnel and health educators who work with parents and adolescents, and focus groups with parents of middle school students and with middle school boys and girls. The key informant interviews and focus groups will be conducted in March of 2006. The results of this formative research are expected to include information regarding recruitment strategies, logistical issues regarding the implementation of the program, and feedback about program content. These results will be used to refine the program content and structure and tailor the program recruitment and implementation to our population of middle school students and their parents. We will use the pilot study to further refine the program, measure the short-term efficacy of the program, and help determine whether to apply for additional funding to run a randomized controlled trial of the program. We plan to conduct the formative work in March of 2006 and so have not yet collected data from which to draw conclusions.

HIV and IDU/Substance Use: Social and Behavioral Risk Factors

Peer-based HIV Prevention among Injection Drug Users and Satellite Syringe Exchangers in California

Presenter: Tom Stopka, California Office of AIDS

Collaborators: K. Irwin, A. Ross

Principal Investigator: Tom Stopka

Background: In California, sharing of contaminated syringes and other injection equipment is linked to 19% of all reported AIDS cases and at least 60% of hepatitis C cases (HCV). Increased access to sterile syringes among injection drug users (IDUs) reduces viral transmission. Syringe exchange programs (SEPs) and pharmacies facilitate access to sterile syringes and increase availability of ancillary health and social services. However, limited hours of service, inadequate geographic coverage, and concerns about visibility during syringe exchange deter many IDUs from using SEPs and pharmacies. IDUs who do not visit SEP sites may nonetheless be receiving their prevention materials and information through networks of satellite syringe exchangers (SSEs) who collect used syringes from their peers, exchange them for new syringes at SEP sites, and deliver them back to their peers. Limited evaluation of the effectiveness of SSE activities in reducing viral transmission risks has been conducted.

Methods: Evaluation activities for this intervention include structured surveys with SSE peer educators at baseline and 3, 6 and 9 month follow-up to learn about SSE demographics, HIV risk behaviors and peer education and SSE activities. Cross-sectional surveys were conducted with SSE clients in order to assess demographics, risk behaviors, services received from SSEs and reasons for depending on SSEs.

Results: To date, the IDU-SSE intervention has enrolled 92 SSEs across 5 counties. Enrolled SSEs have a mean age of 42 years and a mean education level of 11.6 years. Nearly two-thirds (61%) of the SSEs are male and 92% are heterosexual. Sixty-two percent of SSEs are Caucasian, 16% Latino, 15% Native American and 2% African American. Forty-five percent of enrolled SSEs report that they are homeless and 66% have seen a health care provider during the past year. Most enrolled SSEs inject daily (2-3 times) and report injecting an average of 1.9 times with each syringe. Enrolled SSEs have experienced a number of health challenges with 64% reporting that they have had a recent abscess. Further, 26% have had an overdose and 33% report a history of mental illness. Eighty-nine percent of SSEs reported being tested for HIV with 6% reporting receiving a positive result while eighty-three percent reported being tested for HCV and 54% reported receiving a positive result. SSEs reported providing sterile syringes and harm reduction information to more than 10 IDU clients (mean=10.6) in a typical month at baseline and nearly 12 clients at 3-month follow-up (mean=11.75). SSEs also reported talking to their clients about HIV and harm reduction at increased rates after receiving training from project staff.

Conclusion: SSEs appear to fill an important gap in prevention services in California. The new State Office of AIDS intervention is the first to formalize the relationship between SSEs and the public health system. Preliminary findings indicate that this intervention may be effective in extending existing harm reduction services and activities to individuals who are not typically reached.

HIV and IDU/Substance Use: Social and Behavioral Risk Factors

Late Night Breakfast Buffet

Presenter: Valerie Rose, San Francisco Department of Public Health

Principal Investigator: Valerie Rose

UARP Award Number: ID03-SFDPH-013

Background: The Late Night Breakfast Buffet was a feasibility study designed to assess the acceptability and uptake of harm reduction interventions and follow through on referrals among a late night population using a 19' camper van and a 3-person team. The van was parked in a consistent location between 2 and 5 a.m. in the Castro, Polk and South of Market neighborhoods of San Francisco.

Methods: The study had two components: services (i.e., needle exchange, harm reduction counseling, referrals, HIV/STD testing) for the general late night population, and a baseline and 3 month follow up interview targeting 100 men who have sex with men (MSM). The number, estimated age range and apparent ethnicity of individuals accessing services, the types of services and products used by location, and date of delivery were documented. Eligibility for the survey component was 18 and over, male and had sex with men; had anal sex in the last 3 months. Eligible males were invited to be interviewed and were asked to provide substantial contact/locating information for the 3 month follow up. The interview assessed HIV risk behaviors, drug use, and linkages to services. Primary outcome measures were service use, acceptability, preferences and satisfaction with services; behavioral risk profile, referral follow through and prevention impact.

Results: *Services Component.* In a 4-month period, we engaged with over 600 individuals; 2000 needles were exchanged; 20 cases of water/juice and 25 boxes of nutritional snacks and 4500 condoms/lube pillows were dispensed. Twenty-one individuals tested for HIV. Two males were HIV positive; one returned for results, was counseled and linked to HIV care and social services. Six (29%) returned for HIV results disclosure. Twelve males tested for Chlamydia and gonorrhea; 4 returned for results; all results were negative. *Study Component.* We screened 103 men; 73 MSM were eligible; 19 declined to participate primarily due to time constraints. An ethnically diverse sample of 55 MSM enrolled in the study at baseline; 64% identified as gay; 27% bisexual; 6% heterosexual and 4% other. Mean age was 32; 29% were (self-reported) HIV+; 42% were homeless; 25% earned income under the US poverty level. 78% used speed in the last 3 months. Of 65% who ever injected, 97% injected speed; 36% shared needles. Mean number of sexual partners was 9. Despite numerous efforts to locate baseline participants, we located just 31 (56%) for the follow up assessment. Use of stationary needle exchange programs increased and needle-sharing decreased at follow up.

Conclusions: We successfully reached a high risk, late night, disenfranchised population of MSM through mobile harm reduction and demonstrated the feasibility of late night HIV/STD services. HIV/STD testing was less popular; rapid testing might have improved uptake. Mobile needle exchange is an entry point to established needle exchange for MSM-IDU, and should be offered through roving delivery systems. Outreach during the late night hours should be provided to access hard to reach populations.

HIV and IDU/Substance Use: Social and Behavioral Risk Factors

Crystal Methamphetamine and Young Men Who Have Sex with Men: Results from Key Informant Interviews

Presenter: Sharon M. Hudson, Health Research Association, Los Angeles

Collaborators: John Copeland, María Magaña

Principal Investigator: Sharon M. Hudson

UARP Award Number: MU04-HRA-702

Background: Research indicates high rates of unprotected anal sex with multiple partners among young men who have sex with men (YMSM), and highlights the high risk for HIV among this population. This risk is exacerbated by substance use, particularly use of crystal methamphetamine ("crystal"). While this is clearly a population in need of intervention, little data are available on crystal use by YMSM.

Methods: Data were collected as part of formative research for an intervention to reduce or prevent methamphetamine use among YMSM. Seventeen men were recruited from a youth pride festival in Los Angeles. To be eligible, participants had to be between 18 and 24 years old, speak English and either identify as gay/bisexual or report having had sex with another man. Experience using crystal meth was not an eligibility criterion. Semi-structured interviews were conducted using a guide assessing use of crystal meth and other drugs, reasons for using/not using crystal meth, circumstances around initiation of crystal use, norms around crystal use, quitting, and other issues.

Results: Participants' mean (median) age was 21 (23) years. Almost all were men of color (8 Latino, 7 African American, 1 mixed race, 1 White). Five reported a history of homelessness and one reported he was HIV-positive. Virtually all, including those who had never tried crystal (n=4, "non-users"), reported a history of other substance use. Reasons cited for using the drug included to improve sex, to forget problems or escape reality and to stay awake or alert. The most common "downside" mentioned was the physical and emotional pain of coming down off the drug. None mentioned HIV as a risk of crystal use without being specifically prompted about HIV. Non-users tended to say there is no difference between crystal and other drugs. Users were likely to report that at least some users want to quit. It was commonly perceived by almost all participants that users keep their meth use a secret from non-using family and friends. All users (n=13) reported they had tried to quit or reduce their use. Six reported at least 1 month's abstinence from crystal use at the time of interview (range 1 month – 18 months); none had entered drug treatment to quit. Most who quit reported they were sexually active. The influence of family and friends was a key factor in both initiation of crystal use and in efforts to quit.

Conclusions: These data suggest many potential avenues to reduce or prevent crystal meth use among YMSM, and thereby reduce HIV risk. HIV risk assessment and counseling should specifically link meth use and HIV risk, and non-users must be targeted with messages combating the belief that meth is no more dangerous than other commonly used drugs like marijuana. Service providers should assess YMSM for mental health issues and make appropriate referrals to stem the use of crystal meth as self-medication. Drug treatment options tailored to YMSM may be necessary. Interventions to increase social support for quitting or remaining abstinent are particularly important, including crystal-free opportunities to have fun and meet other YMSM.

HIV and IDU/Substance Use: Social and Behavioral Risk Factors

Methamphetamine Use in the U.S./Mexico Border

Presenter: Javier Lopez-Zetina, California State University, Long Beach Foundation

Collaborator: Claire Garrido-Ortega

Principal Investigator: Javier Lopez-Zetina

UARP Award Number: ID04-CSULB-035

Background: The main objective of this study is to describe cross-nationally differences and similarities in patterns of methamphetamine use among individuals in drug treatment in the U.S./Mexico border.

Methods: A cross-sectional in-depth survey with a methamphetamine use instrument was administered to individuals in drug treatment facilities in San Diego, California and the City of Tijuana, Baja California, Mexico. The bi-national survey elicited information on frequency of methamphetamine use before entering treatment, sexual risk behaviors, and cross-cultural ethnic identification. The study includes two phases: phase one; surveying of eligible participants in San Diego, and phase two; surveying of eligible Mexican participants.

Results: Phase One preliminary data from the U.S. side of the study show the following demographic information. Forty-two participants have been interviewed. Sixty-nine percent male, median age 30 years (range 20-49 years), 21% Caucasian, 41% Latino, 5% African-American, and 27% other race/ethnicity. The median age of first use of methamphetamine was 17 years (range 13-41 years), 67% reported smoking as the primary route of methamphetamine administration, followed by snorting (12%), and injecting (7%). Most participants (95%) reported a history of detention. Sexual risk behaviors data indicate that 38% of respondents reported an early initiation into penetrative sex (first experience of this kind occurring at age 15 or younger). While the majority of participants (95%) reported having been tested for HIV, 22% had their last test 2 years or longer prior to the date of interview. A significant cross-national mobility was observed in this sample. One in four participants reported to have lived in Mexico. Of this group 58% reported use of drugs while in Mexico. The spread of methamphetamine use is no longer of concern in the U.S. side of the border; extant data from Mexico indicate that methamphetamine was the main primary drug in Tijuana, Mexico reported by admissions to drug treatment during the period 1996-2002. Further, Mexican women, a group traditionally seen as a low risk population for "hard-core" drug use, are increasingly impacted by methamphetamine abuse. Among admission cases to major drug treatment facilities in Tijuana, Mexico; women were 50% more likely to report methamphetamine use when compared to men.

Conclusions: Methamphetamine use continues to impact extensively the lives of individuals in Southern California and the U.S./Mexico border region. As is the case in the U.S. side of the border, Mexican communities along the border also experience significant challenges associated with the epidemic of methamphetamine use. Mexican cities along the Californian border such as Tijuana and Mexicali report the highest level of methamphetamine abuse in Mexico. With growing economic and social integration between the U.S. and Mexico, significant challenges for the spread of emerging substance abuse epidemics will be faced by the border region. Cross-national efforts and collaboration will be needed to address the impact of these epidemics in the region. Monitoring, preventive, and treatment modalities of these concerted efforts should become integral components of joint cross-national drug abuse programs.

HIV and IDU/Substance Use: Social and Behavioral Risk Factors

Adapting Evidence-based Drug Treatment Interventions for Use as HIV Prevention with Gay and Bisexual Methamphetamine Users

Presenter: Cathy Reback, Friends Research Institute, Inc.

Collaborators: Steven Shoptaw, Kathleen Watt

Principal Investigator: Cathy Reback

UARP Award Number: MU04-FRII-704

Background: Gay and bisexual males who use methamphetamine are at extremely high risk for HIV infection. Methamphetamine use is highly integrated into gay male social contexts, such as circuit parties, sex clubs, and bathhouses. Methamphetamine use in this population, is highly associated with risky sexual behavior, with users of the drug reporting an increased number of sexual partners, decreased use of condoms, and an increased likelihood of being HIV-infected or having a sexually transmitted disease. By contrast, interventions that reduce methamphetamine use among methamphetamine dependent, treatment-seeking gay and bisexual men result in concomitant decreases in HIV-related sexual transmission behaviors that can be measured up to one year following treatment entry. These interventions include contingency management, i.e., provision of increasingly valuable reinforcements for consecutive urine samples that document abstinence from methamphetamine and cognitive behavioral therapy, i.e., a psychological and educational intervention that teaches participants how to initiate abstinence and to prevent relapse. The proposed study will (1) adapt an evidenced-based, gay-specific cognitive behavioral therapy (GCBT) intervention for methamphetamine-abusing gay and bisexual males and transfer its use in a community-based HIV prevention setting; (2) incorporate this GCBT intervention with a contingency management (CM) intervention to yield an optimal behavioral intervention for producing sustained HIV sexual and drug risk reductions; and (3) develop an intervention to provide continuing care to support participants after completion of the intensive, synthetic GCBT+CM intervention with the objective being maintenance of longer-term behavior changes.

Methods: The formative stage of the study included tailoring the evidenced-based GCBT and CM interventions, which received both a scientific and community review. Enrollment began in November 2005. The study will enroll 210 gay or bisexual males who meet criteria for methamphetamine abuse into the 16-week behavioral intervention consisting of GCBT+CM during weeks 1 through 8, and Continuing Care+CM during weeks 9 through 16. Assessments will be conducted at baseline, 8-week, 16-week, and 26-week post admission.

HIV and IDU/Substance Use: Social and Behavioral Risk Factors

Countering a Hidden Risk: Initiating Change among Methamphetamine-using Men Who Have Sex with Men

Presenter: Lorenzo Hinojosa, Office of AIDS Administration, Oakland

Collaborators: Ross Gibson, L. Carver, A. Navarro

Principal Investigator: Lorenzo Hinojosa

UARP Award Number: MU04-OAA-713

Introduction: Alameda County Health Department personnel, community-based HIV and substance abuse service providers, MSM clients and other community stakeholders have collectively acknowledged high rates of methamphetamine use among MSM along with a lack of attention and resources targeting this public health threat. The dangers posed by methamphetamine use are heightened in Alameda County due to the lack of established MSM neighborhoods or districts that can serve as a base for intervention and the ethnic, cultural and geographic nature of the "meth scene". These factors have contributed to a clandestine but extensive underground network of cruising spots, public sex environments and underground sex parties frequented by meth-using MSM. The challenges of gaining access to the Alameda county MSM-methamphetamine "scene" have led to the focus of this project on methamphetamine-using MSM who frequent cruising spots, public sex environments and adult bookstores.

Specific Aims: Based on results of our formative research, we will develop and test a brief outreach-based intervention targeting meth-using MSM that addresses risk reduction with respect to patterns and methods of drug use and sexual behavior. The data gathered will enhance the development of future HIV and substance abuse services for this population in Alameda County. The intervention goals are to 1) initiate relationships between meth-using MSM and health services such as HIV testing and drug treatment; 2) encourage increased use of such services; 3) increase adoption of health protective and harm minimization behaviors. The intervention will consist of four sessions based on the Stages of Change theory and will use motivational interviewing to encourage subjects to establish harm minimization goals related to health, drug and sex behaviors. Adoption of steps toward risk reduction goals will be assessed at three and six months. A Community Advisory Board has been formed in order to gather feedback on our process and results, as well as promote collaboration toward further services for meth-using MSM in Alameda County. A key issue is whether race and culture are associated with patterns of methamphetamine use and adoption of harm minimization behaviors in this geographic area.

Preliminary Results: Preliminary data from our formative research will be presented, including the field observation, ethnographic mapping and an informal analysis of internet sites frequented by meth-using MSM within Alameda County. Individual qualitative interviews are being used to gather formative data from HIV and drug treatment providers, stakeholders such as bar and business owners and meth-using MSM within HIV Care programs, drug treatment programs and the criminal justice system. This data will allow us to refine the intervention prior to pilot testing. Once the intervention is piloted, we will begin full-scale recruitment into the intervention study.

Compendium Only

Network Intervention to Reduce Negative Impact of Methamphetamine Use in Latino MSM

Principal Investigator: Rafael Diaz, California State University, San Francisco

Collaborator: George Ayala

UARP Award Number: MU05-SFSU-501

Background: Latino gay men constitute one of the most vulnerable groups in the nation for the transmission of HIV, showing some of the highest rates of seroprevalence, seroconversion, and unprotected anal intercourse with partners of unknown status. Recent studies of Latino gay men confirm that sexual risk behavior is more likely to occur when under the influence of methamphetamine (MA), with MA users reporting the highest rates (72%) of HIV risk for any Latino MSM subgroup studied to date. Preliminary data suggest that through MA use, men achieve powerful effects – social, psychological, and sexual – that are subjectively and functionally significant in their lives, but often at a big cost to their physical, psychological and sexual well-being. Research to date shows that negative consequences of MA use are related to the user's progressive social isolation, often in response to perceived judgment and stigmatization from partners and friends.

Methods: The purpose of this project is to develop, implement and pilot-test a face-to-face group intervention that trains concerned partners and friends of MA users to intervene in non-judgmental, yet effective ways. Guided by principles of Motivational Interviewing, we will encourage and train participants to interact with their MA using friends in ways that promote self-directed change and access to substance abuse services when appropriate. The intervention will be conducted in Los Angeles and San Francisco and will be guided by principles of Harm Reduction and Motivational Interviewing, and by the sociocultural model of HIV risk among Latino gay men where risk (sexual and substance-related) is understood as shaped by experiences of social discrimination on the basis of race, class and sexual orientation. Intervention development will be conducted in collaboration with members of the target audience, with community-based organizations that serve different segments of the Latino gay men population in each city, and with substance abuse treatment providers.

Results: The presentation will focus on formative research that supports the rationale for the chosen intervention as well as its feasibility and acceptability by concerned partners and friends.

Conclusion: The majority of MA-using men have loved ones who are concerned about their use and abuse, but are not sure how to help. By training concerned friends' and partners' to intervene effectively, we aim to address the problems by enhancing and reinforcing naturally occurring factors of resiliency and strength in the Latino gay community.

Compendium Only

Faith-based HIV Prevention for African American MSM

Principal Investigator: Susan Kegeles, University of California, San Francisco

Collaborators: Greg M. Rebchook, Michael L. Foster, David M. Huebner

UARP Award Number: CR05-SF-721

Background: Young Black men who have sex with men (YBMSM) are at extremely high risk for HIV both in California and throughout the U.S. Our previous research indicated that religion and spirituality are very important in the lives of YBMSM—most were raised in the church and many remain connected to it—yet few HIV prevention interventions have incorporated faith-based approaches into their prevention strategies. In this project, the Unity Fellowship Church Movement (UFCM) and the UCSF Center for AIDS Prevention Studies (CAPS) will collaborate to develop an innovative HIV prevention approach, positioned within a faith-based organization, that will mobilize YBMSM ages 18-29 to reach into the Black community to encourage their peers to have safer sex, obtain HIV testing when needed and, for their HIV-positive peers, to access appropriate medical treatment, if necessary. This project will build on our previous UARP-funded work focusing on developing a community-level prevention model for use in Black AIDS service organizations.

Methods: First, we will conduct 30 semi-structured telephone interviews with representatives of "Open and Affirming" (OA) Black Churches in California that publicly welcome and fully include people of all sexual orientations and identities into their programs, leadership, and ministries. Data from these interviews will assess the capacity, interest and attitudes of OA churches towards HIV prevention. Second, we will conduct 2 focus groups with YBMSM who do not attend church to discuss their attitudes towards OA churches running HIV prevention programs and their beliefs about barriers and facilitators to men who are not affiliated with churches participating in such interventions. Third, UFCM, CAPS, community experts, and consultants will collaborate to develop an HIV prevention intervention for Black faith-based organizations. This third step will involve gaining a better understanding about barriers and facilitators for YBMSM obtaining HIV testing and seeking treatment if they test HIV positive. We will conduct four focus groups: two with HIV-negative men to examine barriers and facilitators to testing and to mobilizing men to encourage their friends to get tested, and two with HIV-positive men to examine concerns about seeking treatment as well as other practical barriers they perceive to accessing health care. Additionally, we will convene two Boards of Cultural Experts, one comprised of YBMSM and the other of older BMSM (i.e., over the age of 30), to provide input on how the intervention should be designed, what components it should have to ensure cultural sensitivity and appropriateness for YBMSM and suitability for use by OA Black churches.

Expected Results: We have not yet collected any data for this project. We anticipate that this study will inform our understanding about YBMSM's spiritual lives that may be complicated by ambivalent relationships with traditional Black churches. Optimally, this spirituality may provide a distinctive leverage point to motivate caretaking both of oneself and of others. Conversely, homophobic messages taught in traditional Black churches may fuel sexual risk taking and dissuade YBMSM from participating in a faith-based program. Thus, we need to better understand Black men's relationships with spirituality and the church before we can effectively integrate prevention into their faith communities.

Conclusions: Not applicable.

California Collaborative HIV/AIDS Research Centers 4

California Research Center, San Diego

California Collaborative Treatment Group (CCTG)

Principal Investigator: Richard Haubrich, University of California, San Diego

UARP Award Number: CH05-SD-607

Since 1986, the California Collaborative Treatment Group (CCTG) has been conducting high impact multi-centered, investigator- initiated clinical trials that address the primary theme of our application: **Emerging Problems in the Management of HIV Infection**. The group has over 140 publications and has a proven record of accomplishments that include: providing access to research opportunities for under represented populations, addressing research questions of importance to HIV infected patients in California, collaborating with diverse disciplines to expand the scope and depth of our research projects, mentoring junior investigators to become the next generation of California clinical investigators, and leveraging funding from additional sources, to build on core funding from the UARP.

The CCTG is a five-center, academic affiliated clinical trials group. The CCTG represents a true partnership between the five sites. All site principal investigators contribute equally to the group leadership process in directing not only the administrative and fiscal matters, but in contributing to the science and management of our studies. Our successful partnership for 18 years is a testament to the collegial, cooperative spirit of the CCTG which is greatly facilitated by monthly face to face meetings in Irvine. At these meetings, we present new concepts, work on existing protocols and discuss new data. Junior investigators are encouraged to lead these discussions which adds to the mentoring process.

The aims and designs of the three proposed studies, addressing emerging HIV management issues, form the core of this application.

Project 1: CCTG 584- Viral dynamics and pharmacokinetics of tenofovir (TDF) and abacavir (ABC). The goal of this study is to determine the pathogenesis of the poor virologic response to TDF + ABC containing regimens. By studying the interaction between TDF and ABC, we can explore the mechanism and determine the viability of this combination. The hypothesis is that the dual NRTI combination will be less potent than either drug used alone and that the difference can be explained in part by an intracellular interaction (reduced levels of intracellular active phosphoralated compounds). The specific primary aims are: to evaluate the relative potencies of TDF or ABC given alone for 7 days compared to TDF + ABC as assessed by the short-term HIV RNA response; and to compare the plasma and intracellular pharmacokinetic data of monotherapy vs. the dual NRTI regimen.

Project 2: CCTG 585- A comparison of once daily Lopinavir/ritonavir (LPV/r) given as liquid versus capsules. The goal of this study is to determine if we can simplify antiretroviral therapy with LPV by evaluating two once daily LPV regimens. Although LPV has been shown to be effective in once daily dosing, diarrhea limits its utility. The hypotheses for this study is that once daily LPV/r liquid will be better tolerated than once daily LPV/r capsules. The primary objective of the study is to compare the tolerability of once daily LPV/r (800/200 mg) given as 10 ml liquid vs. 6 capsules.

Project 3: CCTG 587- Pathogenesis of community-acquired MRSA among HIV + MSM. An important emerging new problem for HIV infected patients is infection with methicillin resistant *Staphylococcus aureus.* Understanding the epidemiology, risk factors and host defenses of this infection will be important to designing strategies to treat and prevent the infections. The objectives of this study are: 1) to prospectively determine the prevalence, incidence, persistence, and risk factors for asymptomatic MRSA colonization among HIV infected MSM and control groups; 2) to compare colonizing MRSA strains with MRSA strains causing clinical infection and to quantify the persistence of strain colonization; and 3) to identify factors in host defenses and host-defense interactions that may prevent *S. aureus* infections.

These studies will address research questions of importance to HIV infected patients in California and will allow the CCTG to continue with our ancillary missions of providing research access to minority patients, mentoring junior investigators and expanding collaborations with diverse disciplines.

California Research Center, Davis

California Research Center for the Biology of HIV in Minorities

Principal Investigator: Richard Pollard, University of California, Davis.

UARP Award Number: CH05-D-606

Investigators at the University of California, Davis School of Medicine with their partner the Viral and Rickettsial Disease Laboratory of the California Department of Health Services are proposing to form a California Research Center for the Biology of HIV in Minorities. The investigators will also focus on gender differences and will conduct specific studies focused on women. The source of patient subjects for the proposed research will be the Center for AIDS Research Education and Services in Sacramento. The investigators feel that detailed biologic studies focusing on immunologic and virologic differences in HIV-infected subjects of different ethnicities and in women are important to describe differences that can be used to tailor therapeutic interventions depending on the results. The clinical site provides HIV care for a diverse patient population which will facilitate the studies as proposed.

The major research project is focused on studies at local versus systemic HIV-specific immune responses and viral diversity. In order to eventually develop vaccines that are targeted at stimulating local protective responses to HIV exposure, understanding of the presence of such responses and how they change over time will be studies. Studies of viral diversity will also be performed to understand whether or not viruses diverge differently in different sites and they change over time. Viruses will be obtained from genital and rectal sites and compared to those obtained in plasma.

The investigators will accomplish these goals through several infrastructure components. An Administrative Core will manage the finances of the Center, perform regulatory and reporting functions, maintain databases and a website and provide statistical support to the Center. A Pilot Project Core will solicit proposals, conduct their review and monitor their progress. The pilot projects will focus on the overall theme of the Center and be funded by UARP resources as well as additional UC Davis resources. A Clinical Core will develop a repository and clinical database on subjects with specimens in the repository. It will also assist Center investigators in the design and conduct of clinical projects. An Immunology Core will provide advanced flow expertise and instrumentation as well as access to a large number of other immunologic assays. A Virology Core located at the partner institution will perform sequencing, tropism assays, resistance assays and other virologic assays required by the Center investigators.

The Center will also enhance HIV research at the partnering institutions, conduct regular seminars and provide training. Important new information about HIV in ethnic minorities and women in California will be developed by this Center.

California Research Center, Los Angeles

Network for AIDS Research in Los Angeles (NARLA)

Principal Investigator: Ronald Mitsuyasu, University of California, Los Angeles

UARP Award Number: CH05-LA-608

NARLA's mission is to support the development and evaluation of innovative strategies for the treatment and prevention of HIV in HIV-infected and at-risk individuals in greater Los Angeles. We propose:

1. To develop and evaluate new and potentially more effective HIV treatment (e.g. hematopoietic stem cell-gene therapy) and prevention (e.g. rectal microbicides) strategies in HIV-infected and at-risk populations.

2. To establish the Network for AIDS Research in Los Angeles (NARLA), a new collaborative group involving UCLA HIV/AIDS researchers, community HIV treatment and service providers and community research organizations.

3. To conduct research that will include and encourage individuals from diverse communities infected or affected by HIV to participate in research of novel strategies to treat and prevent HIV infection.

4. To train and encourage new investigators in cross-disciplinary HIV/AIDS research as it relates to improving treatment, care management and prevention in various populations in California.

5. To conduct pilot studies via seed grant awards to investigators at all NARLA sites to perform exploratory basic, translational, clinical, epidemiological, behavioral and policy studies in areas related to the theme of the Center.

We will accomplish this through development of a strategic interactive partnership between UCLA investigators and community-based treatment and service organizations and through frequent communications, core administration and outreach services and clinical trials infrastructure at each organization. In addition, the Center will maintain a biostatistics core to serve the needs of the two proposed research projects and of the Network investigators. Fellowship awards and seed grants will foster development of new investigators and support exploratory and early developmental work on new projects that fit the overall theme of the Center.

NARLA joins investigators at the UCLA AIDS Institute with investigators and clinicians working at 4 organizations in Los Angeles that provide services and conduct research among diverse populations of HIV infected and at-risk individuals. All 4 organizations have long and distinguished reputations for their service and/or research in the HIV/AIDS community of Los Angeles and all have close working relationships and research collaborations with UCLA AIDS investigators. These organizations include:

- The UCLA CARE Center, an academic clinical research center and HIV clinic which provides both primary and consultative HIV care and which serves as the primary clinical research site of the UCLA AIDS Institute;
- AIDS Project Los Angeles (APLA), a community-based, non-profit organization dedicated to community-based research and to providing education, social support, patient advocacy and educational services to the HIV-infected and –affected communities;
- T.H.E. (To Help Everyone) Clinic, Inc., a community-based clinic in central LA which provides low cost health care to predominantly poorer, minority men, women and families and which has an active HIV clinic that provides medical care, social support and case management services; and
- Friends Research Institute, Inc., a non-profit research organization which conducts research in multiple health areas and which, in Los Angeles, has focused on marginalized and extremely high-risk populations for HIV, e.g. gay and bisexual substance users and male-to-female transgenders.

This network will enlarge the HIV research capacity in Los Angeles and California, ensure that minorities are included in clinical trials, and ensure that the research agenda addresses the needs of disenfranchised community populations. We will build infrastructure at the three non-UCLA sites to allow them to participate actively and collaboratively in the AIDS research efforts. An ongoing two-way interaction between investigators at all partnering sites will occur through monthly phoneconferencing and quarterly face-to-face meetings to discuss research progress and to plan new initiatives. Seed grants and fellowship training awards will be

available to investigators and trainees at all sites to implement new research and further the research capacity at each organization. The research projects proposed in this application will enable the partnering institutions to develop regular procedures for collaborating with UCLA HIV investigators and for participating in multi-center clinical trials. This collaboration will also increase UCLA researchers' ability to include more subjects from diverse populations in their research and broaden the applicability of their findings to a wider group of individuals. We anticipate, as a result of this collaboration, that additional NARLA-based clinical trial of new treatment and prevention interventions for HIV will be possible.

The projects included in this proposal address two emerging and complementary areas of therapeutic and prevention interventions. Project 1 of this proposal focuses on hematopoietic stem cell-gene therapy, a new and exciting area of investigation that holds the potential for reducing or eliminating the need for continuous anti-HIV medication. UCLA is a leader in such cutting edge basic and translational research involving adult stem cells. Because we anticipate even greater interest in the uses of adult and embryonic stem cells and in gene therapy over the next few years, we need to match our basic and clinical research with advances in community understanding of these new technologies and to find ways to encourage participation in clinical trials.

Microbicides are on a fast tract as a new HIV prevention intervention. UCLA is a leader in conducting basic, translational and clinical research on anal-rectal mucosal transmission of HIV, which accounts for over half of all HIV infections in the United States. Project 2 of this proposal will address community knowledge and factors influencing acceptability of rectal microbicides in diverse populations of at-risk individuals, as measured by their willingness to sign up for a registry to participate in future clinical trials of new microbicides. This study will also develop and evaluate the optimal educational interventions to increase willingness of subjects to participate in clinical trials and to accept microbicides as a prevention tool for HIV. We believe both of these projects are timely and involve novel and unique interventions that will require systematic, culturally sensitive and non-judgmental approaches to their evaluation if they are ultimately to become accepted in the community.

California Research Center, Los Angeles

Public Health Consortium for HIV Disparities Research

Pricinpal Investigator: Eric Bing, Charles R. Drew University of Medicine & Science, Los Angeles

UARP Award Number: CH05-DREW-616

Significant disparities in HIV transmission, treatment outcomes and quality of life exist among California's disadvantaged populations. We need a fresh, collaborative approach to developing effective HIV prevention campaigns and treatment programs among these populations.

We believe that the proposed Public Health Consortium for HIV Disparities Research offers that fresh approach. We will promote, conduct and support innovative research on community contexts and social networks that have the potential to reduce HIV-related disparities. This approach holds particular promise for reducing HIV transmission and improving care among communities who often face structural barriers to services and whose cultural beliefs strongly value social, faith-based and friendship networks.

To achieve our goal of stimulating innovative research in HIV disparities, we draw on the unique and complementary strengths of our three partner institutions—the Charles R. Drew University of Medicine and Science, a historically African American medical school; RAND, a public policy and research institute; and the Los Angeles County Division of Public Health, the largest public health department in California. The Consortium will be led by a team with representation from all three institutions, as well as community members impacted by HIV/AIDS.

The Consortium will conduct two original research projects on community contexts and social networks. In the first project, an inter-institutional and multi-disciplinary team will investigate the geographic reach of HIV prevention programs to aid the development of more effective prevention policies and interventions. In the second project, Consortium investigators will evaluate the impact of social support and networks on adherence to treatment in order to develop more effective strategies for keeping African Americans and Latinos with HIV in care.

The Consortium will enhance the research capacity and sustainability of partner institutions to conduct innovative HIV disparities research through strategic planning, project oversight, statistical support, stimulating think tanks, pilot projects, methodological training and support for proposal development.

We believe that our approach has strong potential to invigorate the field through high-quality research and to possibly change the course of HIV/AIDS within disadvantaged and under-researched populations of California.

California Research Center, San Mateo

San Mateo County, San Francisco Peninsula HIV/AIDS Research Center

Principal Investigators: Dennis M. Israelski, Jeff Klausner, David A. Katzenstein

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The overarching theme for the San Mateo County, San Francisco Peninsula HIV/AIDS Research Center is the innovative application of technologies to public health interventions that advance the surveillance, prevention, care and treatment of HIV/AIDS. The partnerships of the San Mateo County, Health Department and Medical Center's Clinical Trials and Research Unit, Stanford's University School of Medicine's Center for AIDS Research, and the San Francisco Department of Public Health's STD Program will promote this theme through interrelated research studies which address problems important to advances in the prevention transmission, pathogenesis, care and treatment of HIV/AIDS. Specific research efforts will focus on (1) evaluation of the detection of early and acute HIV infection as a public health strategy through the development and validation of assays of saliva and oral secretions; (2) providing the foundation for a cohort study to evaluate shedding of HIV in various biological compartments. Informatics and experimental methods in molecular virology will provide new information about the role of drug resistance, fitness and envelope tropism in transmission and pathogenesis. The aim is to understand and improve antiretroviral treatment and secondary prevention strategies in vulnerable patient populations.

Infrastructure components designed to support the Center's theme will build the individual and shared capacity of each partner. This includes strategies to support an emerging community based HIV/ AIDS research center on the San Francisco Peninsula focused on underrepresented populations; create joint mentoring and training infrastructure, to support development of young investigators committed to public health careers in HIV / AIDS research; create a multi-jurisdictional information system, to serve the clinical and research needs of HIV/AIDS patients, providers and researchers; implement community resource strategies, to enroll and retain women and people of color on ART and in clinical trials that are based on principles of participatory community research; award pilot study funding to promising young investigators for projects that advance the Center's research theme and promote the transfer of knowledge, skills and technology.

The Center presents a unique opportunity in California to form a three-way partnership involving two neighboring health jurisdictions. The San Mateo Clinical Research and Trials Unit (CTRU), an emerging research entity with a strong community-based research track-record, will be the coordinating center for this highly innovative partnership that will bring together advanced methods in public health and laboratory science to serve a research agenda that is responsive to the needs of historically understudied populations.

Index of Investigators

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