Pathogenesis
Pathogenesis Working Group Summary

The Pathogenesis working group focused its efforts during WIHS 4 on several different projects. These include evaluation of cellular and soluble biomarkers related to defined WIHS phenotypes (year 1), HCV outcomes (year 2), metabolic outcomes (year 3), correlation with low level viral load determination in HAART suppressed and ELITE controllers (year 4) and neurocognitive outcomes (year 5). The group also oversaw efforts of pharmacology studies and genital tract mucosal immune and viral evaluation. Below is a summary of the results of these studies and publications and presentations resulting from the work.

Cellular Immunology and Microbial Translocation
Seema Desai
Chicago WIHS

The overall WIHS IV pathogenesis aim was to identify key HIV disease phenotypes and assess the extent and patterns of immunological perturbations and to determine whether these immunological characteristics are related to HIV outcomes independently of existing clinical disease markers of HIV disease progression (CD4 cell counts and HIV RNA viral load). We investigated cellular markers of immune activation, cell turnover, apoptosis, exhaustion and senescence, T cell maturation (naive, effector memory, central memory and terminal effectors), regulatory T cell frequencies, T cell function as intracellular cytokine PMA responses and microbial translocation in WIHS defined phenotypes longitudinally - Elite Controllers, HAART controllers, Viremic controllers and HIV negatives as a part of ARRA WIHS supplement. Our key observations from this study, suggests that immune dysregulation persists despite control of viral replication, natural or via HAART and that the pathways to immunological outcomes are disparate. While Elite Control is associated with higher immune activation, viral control via HAART is associated with higher microbial translocation (Desai S MSS in prep). Importantly, we report that Elite women have a phenotype similar to normal aging which is characterized by loss of naïve T cells and accumulation of terminal effectors likely attributed to their higher activation levels and lack of regulation due to low T-reg frequencies compared to HAART Controller/HIV negative.

Other pathogenesis studies were conducted on Elite and HAART controllers. We reported elevated Caspase-3 levels in CD4 and CD8 T cells of Elite controllers and HAART controllers compared to healthy controls suggesting that CD4 decline could occur over period of time via activation induced cell death (AICD) and place individuals at risk of disease progression. To elucidate the cause of immune perturbations in elite controllers, we evaluated low level viral load using TMA assay and found that Elite controllers has higher low level viral replication below<80 copies/ml compared to HAART suppressed women and that they did not differ in genital tract as measured by levels in CVL (Landay Mss in prep). We conducted a cross sectional study to evaluate immune perturbations in HIV/HCV co-infected WIHS women in various WIHS defined phenotypes (Elites, ART controlled, ART uncontrolled and HIV uninfected). Our finding suggests that elite women with HCV viremia have lower Apri scores compared to ART uncontrolled. In the presence of HCV viremia immune activation in Elites persists compared to ART uncontrolled and HIV negative (Desai S, Mss in prep). A pilot investigation was conducted to assess T cell and monocytic activation in HIV infected women with high peripheral fat (Leg fat). Work in progress includes the study on evaluation of role of microbial translocation on cognitive impairment in HIV infected and uninfected women.
Selected Publications and Abstracts


Publications:


Press Citations:


similar to those associated with normal aging - implications for women aging with HIV infection”. Abst. # TUPE072.

Abstract/Presentations from RUSH Cellular and MT Laboratory, Chicago


Chicago Site Specific Peer Reviewed Abstracts and Presentations

Interferon and immune activation, Smoking and Inflammation, HIV/HCV and Microbial translocation


- Desai S, E. Escobar, K. Weber, M. Cohen, A. Landay “Cigarette Smoking Exacerbates HIV Induced Immune Perturbations in HIV+ Treatment Naive Women” : Results from the Women’s Interagency HIV Study(WIHS), Abstract # THPE0098 XVIII International AIDS Conference, Vienna 18-23 July 2010


Soluble Biomarkers
Philip Norris
San Francisco WIHS

Over the past 5 years we have worked with many WIHS investigators. With Dr. Landay we began our biomarker investigation work looking at a set of 39 biomarkers in 4 different comparison groups, HIV non-controllers, HIV negative, and HIV-treated. We found a number of cytokines and chemokines that were elevated in HIV pathogenesis such as IP-10 and TNF-α or lower in loss of immune control such as IL-12p40 and IL-15. In year 2, Dr. Marion Peters and our group focused on studying HIV/HCV co-infection to understand the associations between viral control in co-infection; we found that HCV co-infection increased inflammatory responses including expression of cell
adhension molecules and IP-10. Higher levels of APRI positively correlated with these markers and negatively correlated with CD4 count, IL-17, TGF-alpha and MMP-9. In WIHS year 3, under the direction of Dr. Tien, we performed a number of ELISA assays for the detection of clinical and immune parameters including adiponectin, leptin, IL-6, TNF-a, osteocalcin, C-telopeptide and RANK-ligand. In year 4, again with Dr. Landay, we tested a subset of interesting cytokines, chemokines and growth factors that were identified through the earlier studies to investigate inflammatory responses in individuals with low viral load through treatment or ELITE control. All results have been completed and reported to WDMAC or the investigators. We are currently working on the Year 5 study with Pauline Maki investigating biomarkers of neuropathogenesis. This work will be completed by the end of February. Many studies are currently being analyzed for publication. Although we have not been included in the next phase of WIHS, we will continue to work to complete all analysis and manuscript writing as required. In addition to the 5 WIHS projects we also conducted the ARRA-funded projects which included an extensive study of 72 analytes of over 700 WIHS patient- time points incorporating the groups from year 1 WIHS and ELITE controllers. The cytokines and chemokines from this study that were found to be elevated in ELITE controllers were further investigated in the lab, under the direction of Dr. Evan Jacobs, to identify whether they impact viral infectivity. There is a manuscript currently in preparation summarizing this work. In the second WIHS ARRA-funded study, we studied the impact of long-term HAART therapy in coordination with Robert Kaplan’s studies of the CIMT measurements, immune and cardiac markers. This work has been reported to WDMAC and waiting for analysis. One final ARRA-funded study, for which samples are currently being pulled for testing, investigates the differences in immune responses in HAART responders and non-responders. This work will commence once the samples arrive at BSRI. Finally, the WIHS cohort has provided us with the opportunity to work with a number of WIHS investigators including Deborah Gustafson, Robert Kaplan, Greg Huhn, Toyin Adeyemi, and Kathleen Weber, among others. We have enjoyed the collaborations with the Biomarker working group and WDMAC and we look forward to continuing this work, to complete analysis and publications for all research collaborations.

Publications:


Mucosal Immune and Viral Studies

Betsy Herold
Bronx WIHS

Betsy Herold joined the WIHS Pathogenesis Working Group half-way through WIHS IV after joining the faculty at Einstein-Montefiore. She and her colleagues submitted two concept sheets focusing on soluble mucosal immunity in the genital tract and its relationship to HIV and other viral STI. The first was a cross-sectional comparison conducted among women in WIHS (Visit 33) to explore the hypothesis that compared to HIV-uninfected participants, women with HIV and in particular, those with high plasma viral load (PVL), have increased levels of mucosal and systemic inflammatory mediators and impaired mucosal endogenous antimicrobial activity. Nineteen HIV-uninfected, 40 HIV-infected on antiretroviral therapy (ART) with PVL ≤ 2600 copies/ml (low viral load) (HIV−-LVL), and 19 HIV-infected on or off ART with PVL >10,000 (high viral load) (HIV+−HVL) were evaluated. Immune mediators and viral RNA were quantified in plasma and cervicovaginal lavage (CVL). CVL antimicrobial activity was also determined. Compared to HIV-uninfected, HIV+−HVL women had higher levels of mucosal, but not systemic pro-inflammatory cytokines and chemokines, higher Nugent scores, and lower E. coli bactericidal activity. In contrast, there were no significant
differences between HIV- LVL and HIV-uninfected controls. After adjusting for PVL, HIV genital tract shedding was significantly associated with higher CVL concentrations of IL-6, IL-1β, MIP-1α, and RANTES and higher plasma concentrations of MIP-1α. High PVL was associated with higher CVL levels of IL-1β and RANTES, as well as with higher Nugent scores, lower E. coli bactericidal activity, smoking and lower CD4 counts. Thus, in summary, higher levels of inflammatory cytokines and chemokines in genital tract secretions, lower E. coli bactericidal activity, and higher Nugent scores are associated with higher PVL. These findings suggest that strategies to reduce mucosal inflammation may help control HIV. This work is currently under review at JAIDS and subsequent analyses of data obtained from the same cohort of women with samples from Visits 30-32 is ongoing to examine changes over time.

The second study was a Bronx-site specific study focusing on the biological synergy between HIV and human papillomavirus (HPV). In a study conducted in HIV-uninfected women, we found that women with HPV-associated cervical intraepithelial neoplasia (CIN) had significantly higher levels of pro-inflammatory cytokines in the genital tract compared to healthy PAP-negative controls; changes that could facilitate HIV infection. Building from this work, we are conducting a cross-sectional study within the Bronx/Manhattan WIHS cohort to compare mucosal immunity in HIV infected (HIV+) women with abnormal cervical cytology to those with normal cytology in order to test the hypothesis that HPV infection and an abnormal PAP are associated with changes in the genital tract mucosal immune environment, as well as higher genital tract and plasma HIV viral loads. Preliminary analysis indicates that compared to HIV-infected women with negative PAP test results (n=60), HIV-infected women with low-grade squamous intraepithelial (LGSIL) or atypical squamous cells of undetermined significance (ASCUS) on PAP (n=35) have higher levels of pro-inflammatory cytokines and chemokines in cervicovaginal lavage samples, higher systemic (plasma) HIV viral loads (mean [range] 23,988 [20, 171,000] vs. 7,094 [20, 120,000], (p=0.008) and lower CD4 counts (p=0.002). The increase in inflammatory mediators is associated with significantly greater inhibitory activity of CVL against HSV which may be useful as a future biomarker of inflammation with additional validation. Moreover, the ability of CVL to neutralize HIV infection ex vivo was lower in ASCUS/LGSIL compared to HIV+ women with normal cytology. These preliminary findings support the hypothesis that persistent HPV infection is associated with inflammatory changes in the genital tract, and decreased endogenous anti-HIV activity. The observed changes could facilitate HIV replication and transmission. These findings were presented at the CFAR Joint Symposium on HIV Research in Women in September, 2012 (Providence, RI) and at the 28th International HPV Conference in Puerto Rico in December.

A third WIHS concept to examine impact of hormonal contraception (Depot Provera) on HIV viral loads and explore potential mechanisms by which hormonal contraception may increase HIV mucosal shedding was recently approved; samples have been obtained from the WIHS data base and the studies have been initiated.

Reference:

Pharmacology Studies
Monica Gandhi
San Francisco WIHS

The major pharmacology projects in the WIHS over the past two funding cycles have focused on two major initiatives: the WIHS Intensive PK studies and the WIHS Hair Exposure Studies.

I. WIHS Intensive PK Studies
The genesis of this project arose from the observation that intensive PK studies of ARVs are typically performed for dose-finding purposes after short-term use in the context of phase I clinical trials. These PK studies are usually performed in small, homogeneous populations (sometimes HIV-noninfected) in regards to race/ethnicity, gender and comorbidities. The generalizability of PK evaluations performed in later stage clinical trial settings is also limited due to restrictive eligibility criteria. The typical PK component of clinical trials does not thoroughly investigate the range of individual characteristics (e.g. concurrent medical conditions, dietary patterns, weight differences, ethnicity and gender, use of concomitant medications or recreational drugs) common among patients who will eventually receive ARV prescriptions. The end result can be the revelation of unanticipated adverse effects and treatment failures after drug approval and dissemination.

To address the limitation in the literature and to identify relevant clinical factors that contribute to PK variability in real-world populations, we performed intensive PK studies over 12 to 24 hours for 120 women on each of six ARVs during WIHS IV, specifically nevirapine, efavirenz, atazanavir, lopinavir/ritonavir, tenofovir and raltegravir. Intensive PK data from this large heterogeneous population allowed us to identify a number of clinical factors in multivariate modeling that contribute to increases or decreases in ARV exposure as represented by areas-under-the-curve (AUC), such as hepatic transaminase levels, cocaine use, diet, body mass index, orange juice consumption and renal function. Moreover, we have recently assessed the contribution of genetic traits in addition to non-genetic factors to ARV exposure in the WIHS intensive PK studies by linking with the WIHS Genetics Working Group.

In addition to the rich dataset of intensive PK parameters for large numbers of women in the cohort, and extensive data on possible non-genetic and genetic contributors to exposure, we have been collecting sparse PK levels (single plasma concentrations of the target ARV) at each WIHS study visit. We have now initiated collaborated with an analytic group at UCSF with expertise in nonlinear mixed effects modeling (NONMEM analysis) to assist us in modeling the sparse and intensive PK data in WIHS. We do not plan to perform any additional intensive PK studies of ARVs during WIHS V, but will continue analyzing this unprecedented database of PK and pharmacogenetic data over the next five years, with additional funding to be sought for analytic efforts via linked RO1s.


II. WIHS Hair Exposure Studies

The WIHS UCSF site has pioneered the use of small hair samples to monitor ARV adherence and exposure for patients on combination ARVs after another group presented proof-of-concept analyses that indinavir could be measured in hair samples and correlated with clinical outcomes. We have now developed methods to extract and analyze nucleoside reverse transcriptase inhibitors (NRTIs), prevalent-use protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) from hair and demonstrated that hair concentrations of ARVs are the strongest independent predictor of virologic success in large prospective cohorts of HIV-infected patients in the WIHS. We have also examined factors that contribute to hair concentrations of ARVs in the cohort, including genetic contributors to exposure. This particular exposure project initiated in the WIHS study has now been translated to a number of research settings around the world.
Development of methods to analyze lopinavir (LPV), ritonavir (RTV), atazanavir (ATV), nevirapine (NVP), tenofovir (TFV), and emtricitabine (FTC) in very small samples of human hair by our laboratory using sensitive methods have been developed and reported.\(^5\)\(^-\)\(^8\),\(^13\),\(^14\). We have also developed methods for measuring the HIV protease inhibitor, darunavir (DRV), and the HIV integrase inhibitor, raltegravir (RAL) in hair (manuscript is in preparation) with performance characteristics similar to those we have reported for other ARVs. Analysis of PI, NNRTI and integrase inhibitor levels requires 20-30 strands of human hair, whereas analysis of TFV and FTC requires 50-100 strands.

During WIHS V, we will implement new measures of tenofovir exposure, while maintaining our ongoing measurement of PIs, NNRTIs and integrase inhibitors in hair. For women on TFV-containing regimens, we propose to modify the WIHS protocol to collect 50-100 strands of hair instead of the usual 20 strands. ARV exposure will be assessed as a contributor to key HIV-related outcomes, such as virologic and immunologic responses to therapy, as well as HIV-related outcomes, such as neurocognitive status, bone mineral density, hepatic fibrosis, renal function (especially in relationship to TFV exposure), metabolic outcomes, and vascular injury. Since hair concentrations of ARVs represent an integrated measure of behavior (adherence) and biology (individual pharmacokinetics), we propose that analyses performed in WIHS from this point forward incorporate hair levels of ARVs as markers of adherence and/or exposure. We will also study determinants of ARV exposure using hair measures such as host genetic traits, and develop methods to assess exposure to new HCV medications in hair during WIHS V.


References:
Hepatic fibrosis, immune phenotypes and cytokines vary by HCV viremia in HIV infection.
Marion Peters, Seema Desai and Sheila Keating
San Francisco WIHS and Chicago WIHS

Fibrosis in lymphoid tissue in HIV infection has been attributed to activation of immune-mediated mechanisms, especially enhanced T regs responsiveness. We hypothesized that HCV viremia affects hepatic fibrosis, soluble immune mediators and immune phenotypes in HIV and HCV coinfected women.

We compared elite HIV controllers, HIV uncontrolled on cART and HIV uninfected women. Within groups, we compared HCV RNA positive and negative women, matched by age and race. HCV viremia was associated with higher fibrosis as measured by APRI scores. Higher APRI score significantly correlated with lower %CD4 T cells and a higher % T-reg. HCV viremia was associated with higher IP-10 in Elites. HIV viremia was associated with CD8 T cell activation in HCV RNA+ and HCV RNA women compared to Elites and HIV controlled. However, Elites/HCV RNA- had higher immune activation compared to HIV uninfected/HCV RNA-. In presence of HCV viremia, Elites remain activated compared to HIV controlled and HIV negative.

In HIV/HCV co-infected women, HCV viremia was associated with increased liver Fibrosis. This was ameliorated in women with better HIV control, either natural or via cART. IP-10, an interferon induced protein implicated in liver fibrosis, is increased in the presence of HCV viremia, even in Elite and HIV ART controlled women. HCV driven hepatic fibrosis was associated with lower CD4 numbers and increased T regs while CD8 T cell activation was mainly driven by HIV viral replication.

Presented in Abstract form at Jackson Hole HIV Hepatitis meeting 9-2013, CROI 3-2013 and manuscript being written 2-2013.

Metabolic Studies
Phyllis Tien
San Francisco WIHS

Our work was focused on testing of soluble inflammatory markers and adipokines in women in the WIHS Metabolic Study, as well as testing for low level viremia in Metabolic and Cardiovascular
Substudy participants. We have a planned analysis to examine the association of body composition, adipokines, with bone mineral density. Analysis is also planned to examine whether markers of inflammation and adipokines mediate the association of HIV, HCV, and visceral adiposity with hepatic steatosis.