

## Abstract

**Background:** Human Immunodeficiency Virus (HIV-1) is associated with loss of CD4+ T cells from the peripheral blood, over the course of infection. Gut associated lymphoid tissue (GALT) harbors the majority of CD4+ T lymphocytes in the body and is an important target for HIV. Severe depletion of intestinal CD4+ T cells occurs during primary HIV-1 infection and persists through the course of infection in the absence of anti-retroviral therapy. Inflammation and immune activation continue to be up regulated during Highly Active Anti-Retroviral Therapy (HAART) and are associated with lower levels of CD4+ T cell restoration. Studies suggest that younger HIV-1 infected patients have immune systems similar to uninfected older individuals characterized by a loss of regenerative capacity and an accumulation of aging T cells. However the effects of aging itself on HIV-1 pathogenesis are poorly understood. A growing proportion of HIV-1 infected individuals are now over 50 yrs old. In a chronic inflammatory setting, such as HIV infection, the pathogenic effects of HIV may be exacerbated by the altered hormonal levels during menopause.

**Methods:** Patient groups were analyzed in context of age (men) and hormonal status (women). We evaluated mucosal T lymphocyte subsets and activation using Flow cytometry and immunohistochemistry. Gene expression profiles and viral loads in adult HIV-1 positive patients of all age groups were analyzed using DNA microarray technology and real-time PCR.

**Results:** Immune activation was up regulated during HIV-1 infection and is associated with lower levels of CD4+ T cell restoration in all patient groups during HAART. Altered T cell subsets including central memory T cells and effector memory T cells were observed in post menopausal women. Increased levels of T cell activation were observed in post menopausal women and age matched men in both HIV positive and HIV-1 negative groups. Higher expression of pro-inflammatory factors was observed in CD4+ T cells from older negative controls. Similar results were observed in both HIV-1 positive men and women in the older age group prior to and following the use of HAART.

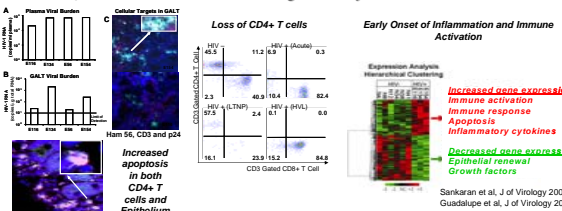
**Conclusions:** Aging results in increased levels of inflammation as well as T cell activation in HIV-1 negative controls and these effects are exacerbated in the presence of HIV infection. The results of this pilot study will be extremely valuable in management of HIV disease worldwide.

## Study Subjects

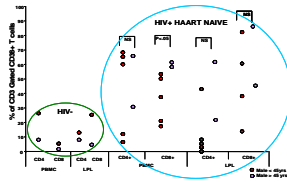
HIV Status	Age Group	Age Range (yrs)	n	# of Subjects	PBMC CD4+ T cells/ml	Average Viral Loads (Plasma)
Male HIV Negative	<45 yrs of age	21-39	4	4	601-1559	N/A
	≥45 yrs of age	50-57	3	3	380-1271	N/A
Female HIV Negative	Pre Menopausal	23-43	5	5	584-1507	N/A
	Post Menopausal	52-59	2	2	510-1418	N/A
Male HIV+ HAART Naïve	<45 yrs of age	21-40	11	11	57-915	4.01E+04
	≥45 yrs of age	49-50	2	2	154-559	2.36E+05
Female HIV+ HAART Naïve	Pre Menopausal	33-49	2	2	471-740	1.19E+04
	Post Menopausal	59	1	1	151	5.50E+04
Male HIV+ Long Term HAART	<45 yrs of age	27-45	7	7	250-1040	UD
	≥45 yrs of age	48-59	7	7	230-1185	UD
Female HIV+ Long Term HAART	Pre Menopausal	39-49	4	4	490-994	UD
	Post Menopausal	51-60	3	3	280-1069	UD

N/A : Not Applicable  
UD: Viral loads were <50 copies/ml

## Rapid Onset of Severe CD4+ T Cell Loss and Disruption of Epithelium Occurs in GALT During Primary HIV Infection



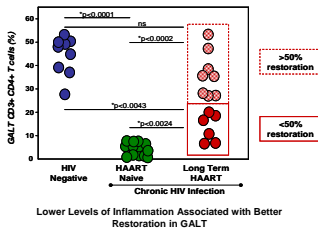
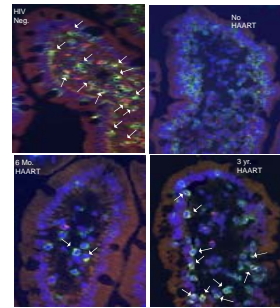
The acute phase of HIV infection occurs in the first few weeks to months following exposure. It is characterized by high viral loads in both peripheral blood and in GALT. The majority of CD4+ T cells are depleted in GALT during this stage of infection and T cell homeostasis is disrupted. All these changes result in the rapid onset of HIV associated enteropathogenesis. (HVL: High Viral Load, LTNP: Long Term Non Progressor)



## Increased T Cell Activation In Chronic HIV Infection

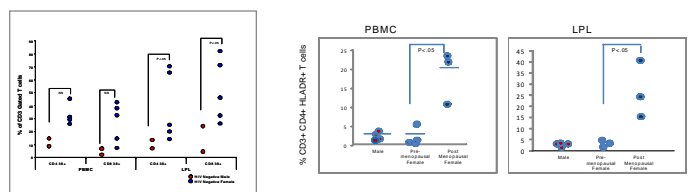
High levels of T cell activation is the hallmark of HIV infection. T cell activation is analyzed using the surface markers CD38 and HLA-DR which are expressed on activated T cells. Significantly higher levels of CD8+ T cell activation are observed in older men with HIV infection.

## Long term HAART can Lead to CD4+ T cell Restoration in GALT of HIV Infected Patients



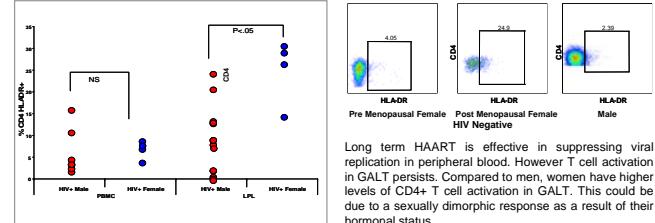
Long-term therapy could reduce, but not fully suppress, HIV RNA levels in the gut mucosa, though substantial gut CD 4 + T-cell restoration was still possible. These data also showed decreased levels of inflammation in GALT during therapy in patients with enhanced CD 4 + T-cell restoration

## Higher Levels of T Cell Activation in HIV Negative Post Menopausal Women



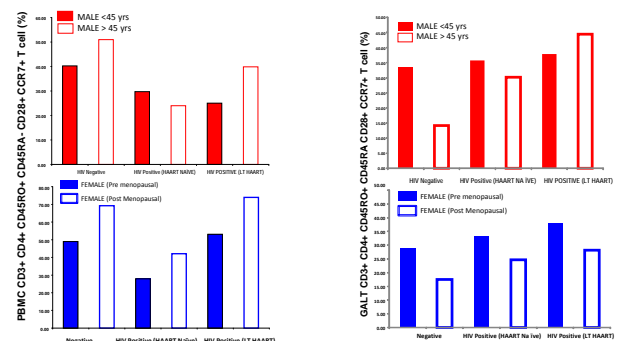
In uninfected controls, women have higher levels of T cell activation in GALT compared to men. Furthermore, post menopausal women have higher levels of CD4+ T cell activation in peripheral blood as well as in GALT

## T cell Activation in GALT is Maintained in Women Despite Long Term HAART



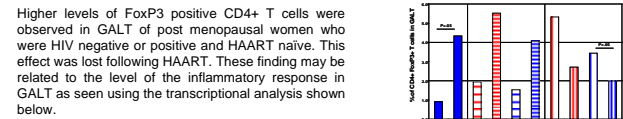
Long term HAART is effective in suppressing viral replication in peripheral blood. However T cell activation in GALT persists. Compared to men, women have higher levels of CD4+ T cell activation in GALT. This could be due to a sexually dimorphic response as a result of their hormonal status.

## Central Memory T cells are Lower in Post Menopausal Women



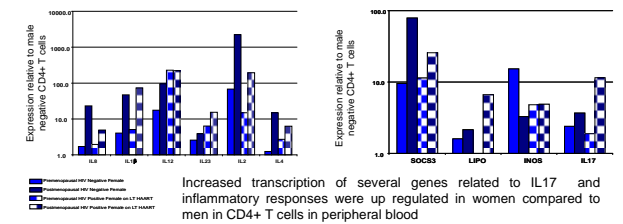
Lower levels of Central Memory T cells were observed in GALT in both men and women of older age groups who were HIV negative or positive. These lower levels were maintained during the course of HAART in post menopausal women. This is in contrast to observations in peripheral blood.

## Regulatory T cell levels maintained in at Higher Levels in GALT in Post Menopausal Women in the Absence of Long Term HAART



Higher levels of FoxP3 positive CD4+ T cells were observed in GALT of post menopausal women who were HIV negative or positive and HAART naïve. This effect was lost following HAART. These findings may be related to the level of the inflammatory response in GALT as seen using the transcriptional analysis shown below.

## Transcriptional Up regulation of pro-inflammatory/pro-activation genes in Post Menopausal Women



Increased transcription of several genes related to IL17 and inflammatory responses were up regulated in women compared to men in CD4+ T cells in peripheral blood

## Conclusions

- HIV infection results in CD4+ T cell depletion in GALT
- Progression is associated with T cell activation and tissue inflammation
- Partial T cell restoration occurs with HAART
- A threshold of CD4+ T cell restoration correlates with:
  - Reduced immune activation in GALT
  - Reduced inflammation in GALT
  - Suppression of viral replication

Persistence of the immune activation despite the viral suppression  
Aging is associated with higher levels of T cell activation in women  
Effects of menopause on inflammation and immune activation plays an important role in HIV pathogenesis

## Acknowledgements

We thank UCDMC GI and J St Clinic staff, CARES, California HIV AIDS Research Program CH5-D-606, Dr Jerome Braun for statistical advice, patients for their participation and Tasha Santos and Lynell Clancy for assistance with patients. Study support was provided by the National Institute of Health (NIH) R01 DK61297 and California HIV AIDS research program CH05-D-606. We also thank the Lucy Whittier Molecular Core and the School of Medicine Microarray Core for technical support.