



HIV-1 Infected Long Term Non-Progressors Display Normal CD4+ T Cell Levels and Reduced Immune Activation Within the Intestinal Mucosa

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Abstract

Background: Limited information is available on the molecular mechanisms characterizing the stages of HIV-1 infection. Furthermore the mechanisms by which some individuals, called long-term HIV-1 infected non-progressors (LTNP), naturally suppress HIV-1 infection and maintain immune functions is unknown. The mucosal immune system is an early target for HIV-1 infection and severe CD4+ T cell depletion. These changes occur early in infection and are maintained through the course of infection in the absence of anti-retroviral therapy. The pathogenic mechanisms introduced early in infection in the microenvironment of the gut is also largely unknown.

Methods: We evaluated mucosal T lymphocyte subsets, virus-specific responses, gene expression profiles and viral loads in intestinal mucosal biopsies of patients in primary HIV-1 infection, LTNP patients and untreated chronically HIV-1 infected patients with high viral loads and CD4+ T cell loss (HVL) as compared to HIV-seronegative healthy individuals. The goals of the study were to identify the mucosal molecular correlates of stages of HIV disease progression and to determine the molecular changes associated with immune and intestinal dysfunction. Mucosal gene expression profiles were studied using DNA microarray technology, tissue viral loads were monitored using Real Time PCR and immunophenotypic analysis of peripheral blood mononuclear cells and gut lymphocytes were performed using flow cytometry and immunohistochemistry.

Results: While LTNP patients had undetectable viral loads and normal CD4+ T cell levels in peripheral blood and mucosal compartments, patients in the early stages of HIV 1 infection had detectable viral loads in the gut as well as a pronounced CD4+ T cell depletion. Oligonucleotide microarray analysis revealed a significant increase in gene expression regulating immune activation, cell trafficking, and inflammatory response in intestinal mucosa of HVL patients and patients in early stages of infection as compared to LTNP patients. Genes associated with cell cycle progression were similarly dysregulated in all patient groups. Alterations in gene expression that were common to early patients and LTNP patients included a group of genes with common upstream transcription factors. Interestingly, genes associated with lipid metabolism and epithelial cell barrier and digestive functions were down regulated in both HVL and LTNP patients and to varying degrees in early infection. This may adversely influence nutrient adsorption and digestive functions, with the potential to impact the efficacy of anti-retroviral therapy.

Conclusions: Our findings indicate that, in contrast to HVL patients, LTNP patients maintain both peripheral and gut CD4+ T cell levels and control immune and inflammatory responses. Down regulation of genes associated with digestive and barrier functions highlights the insidious effects of HIV-1 infection that occur in patients in all stages of disease progression, regardless of their ability to control viral replication

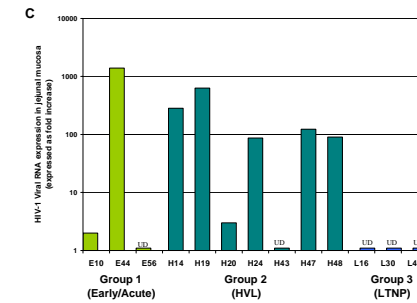
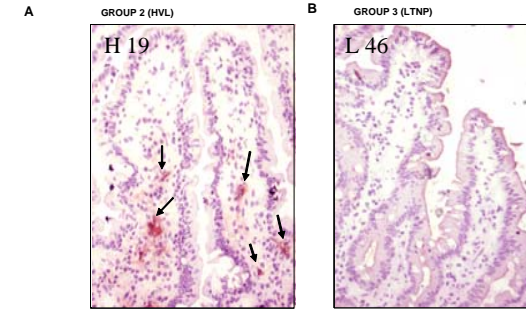
Study subjects and sample collection

Sixteen ART-naïve HIV-1 sero-positive individuals and 7 HIV-1 sero-negative healthy individuals were enrolled in the study. Endoscopic jejunal biopsy specimens were cryo-preserved for transcriptional analysis and immunohistochemistry, or collected in RPMI 1640 for flow cytometric analysis. Peripheral blood samples were collected at the time of biopsy. The Institutional Review Board at University of California, Davis, CA, approved the study and written informed consent was obtained from all participants in the study.

Range of CD4+ T cell counts in patient study groups.

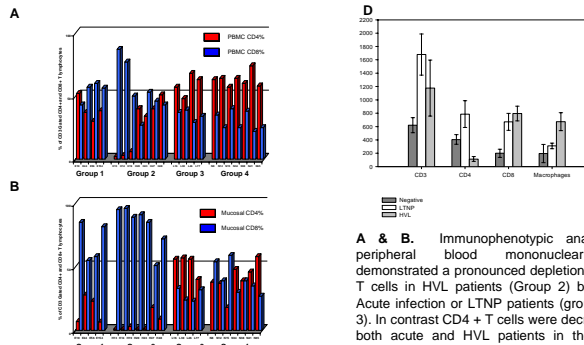
Study Group	Pt ID	Age	CDC Classification	Duration of infection	Peripheral CD4+ T cell counts
EARLY HIV INFECTION GROUP 1	E10	34	A1	6 wks	881
	E44	35	A1	4 wks	752
	E56	33	A1	4 wks	510
	E154	32	A1	8 wks	915
HIGH VIRAL LOAD GROUP 2	H13	48	C3	1 yr	8
	H14	36	C3	1 yr	6
	H19	35	C3	1 yr	46
	H20	45	C3	1 yr	294
	H24	34	C3	1 yr	34
	H43	30	C2	1 yr	174
LONG TERM NON PROGRESSOR GROUP 3	H47	23	C1	1 yr	205
	H48	42	C1	1 yr	427
	L16	49	A1	12 yrs	910
	L30	43	A1	7 yrs	587
HIV-1 SERONEGATIVE GROUP 4	L46	45	A1	17 yrs	787
	L77	40	A1	8 yrs	968
HIV-1 SERONEGATIVE GROUP 4	N8	22	NA	NA	1046
	N12	22	NA	NA	1236
	N15	52	NA	NA	ND
	N34	35	NA	NA	1046
	N38	48	NA	NA	632
	N41	43	NA	NA	1203
N65	37	NA	NA	634	

HIV-1 RNA is undetectable in the jejunal mucosa of long term non-progressors



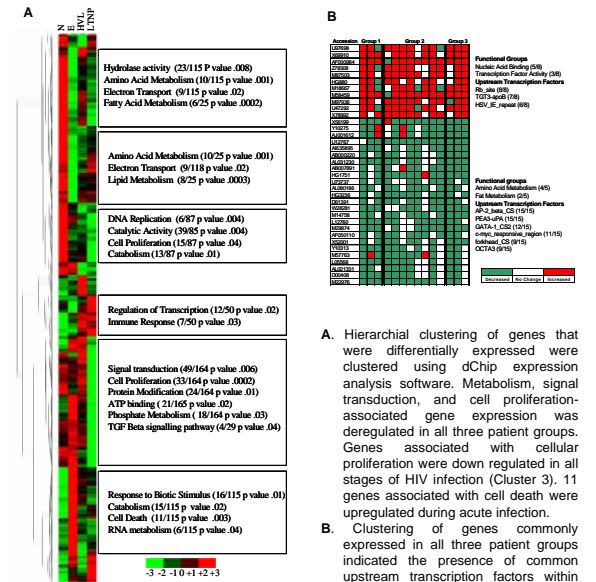
A & B. Viral HIV-1 p24 was detected in the villi of patients with chronic HIV infection (Group 2) but not in long term non progressors (Group 3) (Arrows indicate areas of HIV-1 p24 expression demonstrated using immunohistochemistry).
C. Viral RNA was detected in all patient groups except LTNP patients using real time (Tagman) PCR. (UD = below the limit of detection).

HIV-1 infection induces dramatic alterations in T cell subsets during all stages of infection



A & B. Immunophenotypic analysis of peripheral blood mononuclear cells demonstrated a pronounced depletion in CD4+ T cells in HVL patients (Group 2) but not in Acute infection or LTNP patients (group 1 and 3). In contrast CD4+ T cells were decreased in both acute and HVL patients in the jejunal mucosa accompanied by an increase in CD8+ T cells.
C. HAM 56 staining for tissue macrophage demonstrated significant increase in numbers of macrophages in HVL patients.
D. CD4+ T cells were severely depleted from the jejunal mucosa of HVL patients, coinciding with an increase in CD8+ T cells. Counts of Ham 56+ cell indicated increased numbers of macrophages in the intestinal mucosa of both LTNP and HVL patients.

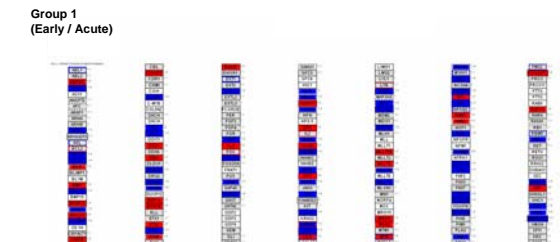
Metabolism associated gene expression was decreased while immune function associated gene expression was increased in all stages of HIV infection



A. Hierarchical clustering of genes that were differentially expressed were clustered using dChip expression analysis software. Metabolism, signal transduction, and cell proliferation-associated gene expression was down regulated in all three patient groups. Genes associated with cellular proliferation were down regulated in all stages of HIV infection (Cluster 3). 11 genes associated with cell death were upregulated during acute infection.
B. Clustering of genes commonly expressed in all three patient groups indicated the presence of common upstream transcription factors within the upregulated and down regulated clusters.

Molecular mechanisms of HIV-1 associated enteropathy

Cellular growth and differentiation associated gene expression is dysregulated early in HIV-1 infection



HIV-1 infection results in disruption of the mucosal microenvironment. The molecular mechanisms resulting in these changes are not well defined. Our data suggests that dysregulation of cell growth and differentiation along with the immune and inflammatory reactions evoked by the presence of virus may contribute to this epithelial and immune dysfunction.

Acknowledgements

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