HIV-1 Infection Leads to Increased Expression of HML-2 Proviruses In Vivo but not Virion Production

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BACKGROUND



- Endogenous retroviruses make up ~8% of the human genome.
- The HERV-K (HML-2) group includes the most recently integrated proviruses and are present as both fixed and polymorphic loci in the human population. Some of these proviruses have retained ORFs for the retroviral genes *gag*, *pro*, *pol* and *env*.
- HML-2 provirus expression (in the form of RNA, proteins and viral particles) is found in a variety of human disease states, including cancers and autoimmune diseases. Recently, studies identified HML-2 expression in HIV-1 infection. Patients were shown to exhibit an immune response against HERV antigens and HML-2 viral RNA was detected in HIV-1+ plasma and PBMC samples.

EXPERIMENTAL DESIGN

- The purpose of this study is to confirm if HML-2 expression in upregulated in HIV-1 infected plasma and PBMCs and, if so, to determine the source of HML-2 expression. Additional analyses focus on the relationship of HIV disease markers to HML-2 expression in HIV-infected patients.
- HML-2 RNA copy number is quantified using a SYBR-based quantitative PCR that is capable of detecting 51 copies of HML-2 provirus per haploid genome. RNA was left untreated or treated with DNase prior to reverse transcription and detection using the HML-2 *env* qPCR.
- **Viral RNA Extraction**: Virions were pelleted from patient plasma, digested with Proteinase K and lysed with guanidinium isothiocyanate. RNA was pelleted and resuspended in 5mM Tris-HCl. RNA was left untreated or DNase-treated prior to analysis.
- **PBMC RNA Extraction**: PBMCs were washed in PBS and pelleted. RNA was extracted using a TRIzol-chloroform extraction and eluted using a PureLink column kit (Invitrogen). RNA was always DNase treated.
- **PBMC Sorting and RNA Extraction**: Live patient PBMCs were sorted into the major cell subsets (CD3+CD4+ T cells, CD3+CD8+ T cells, CD3-CD14+ monocytes and CD3-CD20+ B cells) using specific antibodies. Cells were sorted into PBS and kept on ice prior to RNA extraction using a column kit (Qiagen). RNA was always DNase treated.

PATIENT CHARACTERISTICS

Table 1. HIV-infected patient characteristics for plasma and PBMCs analyzed in Figures 1-3.

NIH Clinical Center and TMC*	Plasma = 15		PBMC = 19	
	n	%	n	%
Demographic Characteristics				
Male	14	93	18	95
	6	40	9	47
African-American	4	27	3	16
Hispanic	5	33	6	32
Asian Pacific-Islander	0	0	1	5
	Median	Range	Median	Range
Age (years)	42	25.1 – 48.0	35.9	19.5 – 51.9
HIV Characteristics				
CD4 Count	523	220 – 1105	661	210 – 1105
%CD4 Cells	24.8	10.7 – 55.7	29	9 – 55.7
RNA Viral Load (log_{10} copies/mL)	4.2	1.95 – 5.6	4.11	1.95 – 5.55

Note: One viremic HIV-infected patient was off-therapy during blood collection. All other patients are treatment-naïve and viremic.
*TMC, Tufts Medical Center.

Table 2. HIV-infected patient characteristics for PBMCs analyzed in Figure 4.

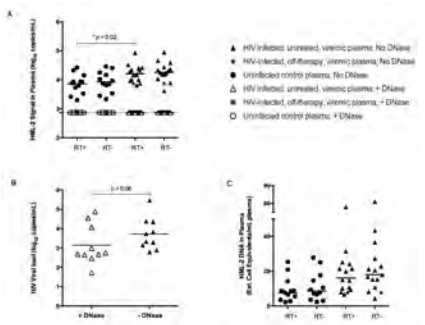
UPitt*	Non-Viremic = 10		Viremic = 5	
	n	%	n	%
Demographic Characteristics				
Male	7	70	5	100
White	3	30	0	0
African-American	7	70	4	80
Hispanic	0	0	1	20
Asian Pacific-Islander	0	0	0	0
	Median	Range	Median	Range
Age (years)	51.5	25 – 58	52	29 – 53
HIV Characteristics				
CD4 Count	617	416 – 1373	874	244 – 1091
%CD4 Cells	31.1	20.1 – 52.1	31.9	9.3 – 47.4
iSCA** Viral Load (log_{10} copies/mL)	N/A	N/A	3.44	2.75 – 5.32

Note: All non-viremic patients were on therapy. Viremic patients were either treatment-naïve, off-therapy, or on therapy at the time of blood collection.
*University of Pittsburgh Medical Center
**iSCA is a modified single copy assay (SCA), detecting HIV integrase.

- All patients analyzed do not have history of medical conditions associated with HML-2 expression (cancer, autoimmune disease, schizophrenia) and were not pregnant. The control uninfected plasma and PBMCs are from healthy volunteers, age 18-65.

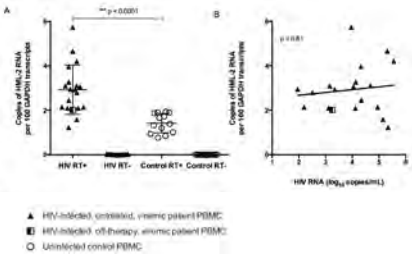
RESULTS

FIGURE 1: HML-2 SIGNAL IN PLASMA RNA +/- DNASE



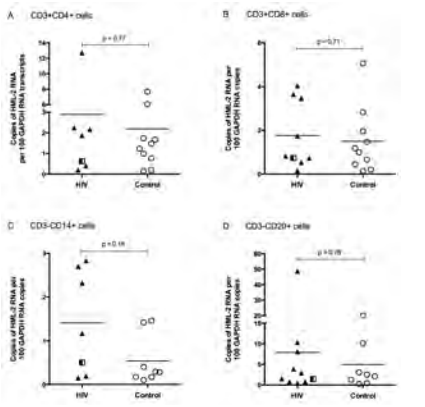
(A) HML-2 virus was not detected in control or HIV-infected patient plasma RNA treated with DNase. In RNA w/o DNase treatment, RT+ = RT- signal. Dotted line at limit of detection (730 cps/ml). *p=0.02 from Mann-Whitney *t*-test. (B) HIV was detected in patient RNA with or without DNase. p=0.06 from Wilcoxon signed rank test. (C) Estimated number of lysed per cells/ml plasma contributing to HML-2 DNA signal. Geometric means (A-B) or means (C) plotted for each group.

FIGURE 2: HML-2 RNA EXPRESSION IN HIV-INFECTED PATIENT PBMCs



(A) HML-2 RNA was upregulated relative to GAPDH expression in HIV-1 infected PBMCs. Mean and standard deviation are plotted. ***p<0.0001 from Mann-Whitney *t*-test. (B) Upregulation was not correlated with HIV plasma viral load (B), CD4 counts or %CD4 cells in the tested patient s. P value for linear regression shown.

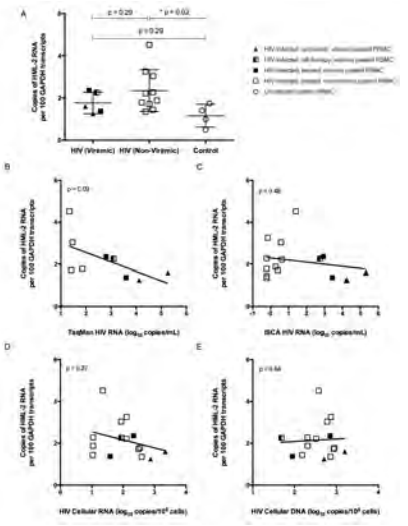
FIGURE 3: HML-2 RNA EXPRESSION IN MAJOR PBMC SUBSETS



PBMCs from viremic HIV-infected patients and uninfected controls were stained with antibodies specific to CD3, CD4, CD8, CD14 and CD20. HML-2 RNA expression was analyzed in sorted live cells that were (A) CD3+CD4+ T cells, (B) CD3+CD8+ T cells, (C) CD3-CD14+ monocytes and (D) CD3-CD20+ B cells. Means are plotted for each group and p values for Mann-Whitney *t*-tests are shown. Refer to the Figure 2 legend for sample descriptions.

RESULTS

FIGURE 4: HML-2 RNA EXPRESSION IN PBMCs FROM HIV-INFECTED PATIENTS ON THERAPY



(A) HML-2 RNA was quantitated in PBMCs collected from HIV-1 infected subjects on antiretroviral therapy without detectable viremia, from patients with viremia who were either on therapy, currently off therapy, or naïve to therapy and from uninfected controls. Open symbols indicate samples with undetectable HIV RNA (<75 cps/ml). (*p=0.02, Mann-Whitney). Mean and standard deviation are plotted for each group. (B-E) The level of HML-2 RNA expression in PBMCs is plotted against (B) HIV plasma RNA measured by TaqMan assay, (C) HIV plasma RNA measured by iSCA assay, (D) cellular HIV RNA, or (E) cellular HIV DNA. The p value for linear regression is shown in the upper left hand corner.

CONCLUSIONS & FUTURE DIRECTIONS

- **HML-2 viral RNA is not detected in the plasma of HIV-1 infected patients.**

Potentially, HML-2 virions are not expressed in HIV-1 infection, HML-2 viral RNA is below the limit of detection or HML-2 virions are expressed in a distinct subset of patients.

- **HML-2 RNA expression is upregulated in PBMCs relative to GAPDH.**

RNA upregulation did not appear to be related to HIV disease markers in this study. Accordingly, patients with and without viremia showed upregulated HML-2 RNA. The mechanism governing expression may be due to an indirect effect of HIV infection, such as immune activation. The exact proviruses expressed in HIV-infected patients are being investigated in addition to the possible effects increased HML-2 expression may have on disease progression, such as malignancy.

- **HML-2 RNA expression is not enriched in a PBMC cell subset.**

The major subsets tested did not show enrichment for HML-2 RNA in HIV-infected patients. It is plausible that an untested cell subset (like NK cells and dendritic cells) or a minor cell population in the subsets tested are involved but undetectable by our assay.

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