Transcripts Associated with the HIV Reservoir Correlate with the Pool Size of Long-lived CD4 T Cells

Maria J. Buzon[1,2], Zhengyu Ouyang[2], Amy Shaw[2], Forencia Pareyra [2,3], Judith Dalmau[4], Nadine Zangger[5], Javier Martinez-Picado[4,6], Xu G. Yu[2], Amalio Telenti[5], Eric S. Rosenberg[1], Mathias Lichterfeld[1] .

[1]Infectious Disease Division, Massachusetts General Hospital, Boston, MA; [2]Ragon Institute of MGH, MIT and Harvard, Boston, MA; [3]Infectious Disease Division, Brigham and Women's Hospital, Boston, MA, [4]Institut de Recerca de la SIDA IrsiCaixa, Hospital Germans Trias i Pujol, UAB, Badalona, Spain

[5]Institute of Microbiology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [6]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Background

Although active HIV-1 replication can be effectively suppressed by antiretroviral therapy (ART), the virus persists despite treatment and rapidly rebounds once treatment is discontinued. Prolonged ART initiated early after infection has been shown to reduce the size of the HIV-1 reservoir, however it does not eradicate the viral infection. This long-term viral persistence is mainly due to the presence of latently infected CD4 T cells, but host factors that modulate the size of the latent viral reservoir are incompletely understood.

Hypothesis: The viral reservoir is maintained by specific pathways that regulate homeostasis of long-lived CD4 T cells

Methods

CD4+ cells were sorted after a median of 10 years of undetectable viremia from 37 treatment-naive Elite Controllers (EC), 10 patients who initiated ART in the chronic phase of infection (CT) and 8 patients initiating ART within 90 days of infection (ET). Total HIV-1 DNA was determined by qPCR and following mRNA extraction, whole-genome transcriptional profiling was performed using Illumina HumanHT-12 microarrays according to standard procedures and differentially expressed genes were defined as having >1.5-fold differences in gene expression intensity and an FDR-corrected p-value of < 0.05. A linear regression model in R was used to identify gene transcripts associated with viral reservoir size in CD4 T.

Results

Analysis of total HIV-1 DNA between our different cohorts showed that HIV-1 infected CD4 T cells exhibit lower reservoirs in EC and after long-term ART initiated in early HIV-1 infection compared to long-term ART initiated at chronic HIV-1 infection (Figure 1).

Figure 1

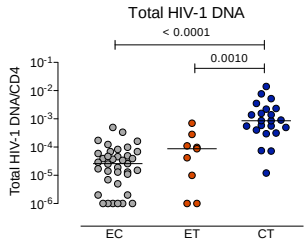


Figure 1. Copies per cell of total HIV-1 DNA in isolated CD4 T cells from individuals started on suppressive antiretroviral therapy in early HIV-1 infection (ET), chronic HIV-1 infection (CT) or from elite controllers (EC). Study patients have undetectable viremia for >8 years (EC) or >10 years (ET/CT).

To better understand the mechanisms responsible for the maintenance of the viral reservoir in CD4 T cells for >10 years of continuous antiretroviral therapy, we analyzed gene expression patterns of total CD4 T cells. We found that a total of 215 transcripts were differentially expressed between individuals who started antiretroviral therapy in early versus chronic HIV-1 infection (>1.5 fold difference, FDR-adjusted p<0.05). Using the same criteria, we found that 211 of these transcripts were also differentially expressed between elite controllers and persons who started treatment in chronic HIV-1 infection, while only three transcripts were differentially expressed between elite controllers and persons started on treatment in early HIV-1 infection (Figure 2A).

An unsupervised hierarchical linkage clustering analysis demonstrated that based on expression patterns of these 215 transcripts, many of the individuals started on treatment in early HIV-1 infection clustered with elite controllers, while gene expression profiles in persons with treatment initiation in chronic infection were more distinct (Figure 2B)

Figure 2

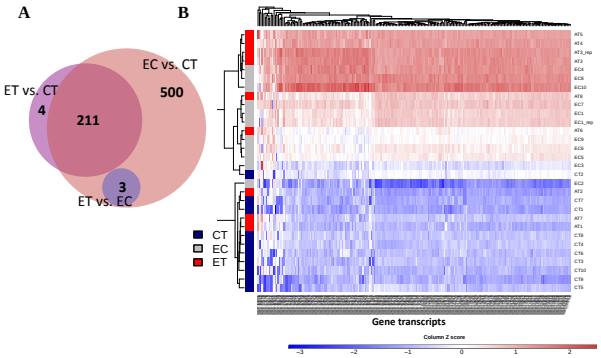
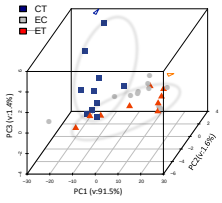


Figure 2. (A) Three-circle Venn diagram reflecting the numbers of differentially expressed genes. (B) Transcripts that were differentially expressed between individuals started on treatment in primary vs chronic HIV-1 infection and elite controllers.

In addition, principal component analysis also suggested that long-term antiretroviral therapy initiated in primary HIV-1 infection induced gene expression patterns in CD4 T cells that closely resemble those of elite controllers (Figure 3)

Figure 3



Notably, linear regression analysis demonstrated that 211 of the differentially-expressed transcripts between patients treated in early versus chronic HIV-1 infection were negatively correlated to the total viral reservoir size (HIV-1 DNA copies/cell) in total CD4 T cells (each at an FDR-corrected p<0.05). Many of the transcripts associated with the viral reservoir size in total CD4 T cells were also significantly correlated to the relative proportion of CD4 T memory stem cells and of central-memory cells within the total CD4 T cell pool (Figure 4), but not with any other tested immunological characteristics of the patients, including total CD4 T cell count or levels of immune activation in CD4 or CD8 T cells (determined by HLA-DR surface expression).

Figure 4

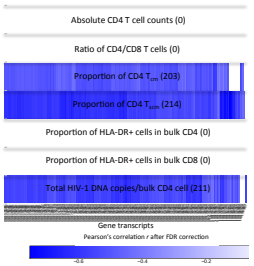


Figure 4. Linear regression analysis demonstrating associations between n=215 differentially expressed gene transcripts and indicated immunological and virological characteristics of study patients. Significant associations (FDR-corrected p<0.05) are highlighted; numbers of transcripts with significant associations are indicated in parentheses. Color-coding reflects strength of association (r)

Conclusions

Gene transcripts associated with the size of the viral reservoir frequently correlated with the pool size of the long-lived CD4 T cells, suggesting that maintenance of the viral reservoir is guided by shared molecular pathways that also regulate homeostasis and pool size of these long-lasting T cell populations. This suggests that specific therapeutic intervention targeting the most durable CD4 T cell subsets will likely be necessary to reduce HIV-1 persistence in a clinically significant way.

Acknowledgements

This work was supported by the US National Institutes of Health (grants AI098487 and AI106468 to M.L., AI089339 to X.G.Y., AI100699 to J.Z.L.). M.L. is a recipient of the Clinical Scientist Development Award from the Doris Duke Charitable Foundation (grant number 2009034). M.J.B. is supported by the Tosteson postdoctoral fellowship award from Massachusetts General Hospital. Patient blood sample collection was supported by the US National Institutes of Health (grant AI074415), by the Mark and Lisa Schwartz Foundation and by the Bill &Melinda Gates Foundation.

)