Short Communication: Expression of Host Restriction Factors by Memory CD4+ T Cells Differs Between Healthy Donors and HIV-1-Infected Individuals with Effective Antiretroviral Therapy

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Abstract

Much has been learnt from the functions of host restriction factors during acute and chronic HIV-1 infection, but far less is known about their role in HIV-1-infected individuals in which viral load is stably suppressed with antiretroviral therapy (ART). In this study transcriptional expression of 42 host restriction factors was determined for memory CD4+ T cells sorted from 10 uninfected and 21 HIV-1-infected individuals, treated with suppressive ART and for which the viral reservoir was quantified. No significant associations were observed between restriction factor expression and HIV-1 reservoir size, quantified by measurement of HIV-1 Gag DNA using droplet digital polymerase chain reaction, and by measurement of replication-competent inducible virus using quantitative viral outgrowth assays. Expression of eight of the restriction factors differed significantly, and with a false discovery rate of <10%, between ART-suppressed and uninfected individuals. APOBEC3G, ISG15, LGALS3BP, RNASEL, and MX2 were upregulated in the ART-suppressed individuals, likely because of increased levels of immune activation observed in virally suppressed compared with uninfected individuals. In contrast CDKN1A, TRIM11, and BRD4 were expressed at lower levels in ART-suppressed than uninfected individuals. This suggests perturbation of the CD4+ memory T cell compartment, in which a viral reservoir persists in HIV-1-infected individuals with effective ART. Modulation of restriction factor expression, or overrepresentation of cell subsets that intrinsically express these restriction factors at lower levels could result in the distinct expression of restriction factors observed in treated infected individuals.

Keywords: HIV, viral reservoirs, restriction factors, antiretroviral therapy, persistent infection

Introduction

IN HIV-1-INFECTED INDIVIDUALS treated with effective antiretroviral therapy (ART), a reservoir of latently infected cells results in viral rebound if ART is discontinued. This reservoir can be quantified, using polymerase chain reaction (PCR) or *ex vivo* viral outgrowth assays, and has been found to derive predominantly from memory CD4+ T cells.¹ The low frequency of latently infected cells has prohibited determination *in vivo* of their phenotypic characteristics that could shed light on the mechanism of reservoir persistence and enable targeting of this population for therapeutic cure. Host restriction factors are cell-intrinsic proteins that inhibit viral replication and are typically upregulated in response to interferons. Many are upregulated in acute and chronic HIV-1 in-

fection, and they have also been found to be expressed at higher levels in elite controllers and B*57+ individuals.^{2,3} Several host restriction factors directly affect HIV-1, such as IFITM1 that inhibits *in vitro* infection and BST2 that is antagonized by the viral accessory protein Vpu. Here we characterize expression of host restriction factors in virally suppressed individuals to potentially indicate changes in the composition of the memory CD4+ T cell compartment of these individuals compared with uninfected controls, and because these infection-induced genes also represent candidate markers for latently infected cells. Transcriptional expression of 42 host restriction factors was determined for memory CD4+ T cells from 21 HIV-1-infected individuals with suppressive ART, for which the viral reservoir was quantified by measurement of viral DNA and replication-competent inducible virus, as well as for 10 uninfected donors.

We identified a distinct phenotype of restriction factor expression in these primary samples from ART-suppressed individuals.

Methods

Study participants were recruited by the “Reservoir Characterization Support Section” of the BELIEVE consortium (USA), and by the Maple Leaf Medical Clinic (Canada), through protocols approved by their respective institutional review boards. All donors provided written informed consent. HIV-infected individuals had viral loads stably suppressed by ART for at least 6 months before sample collection, and CD4/8 counts at the time of analysis are detailed in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/aid).

Cryopreserved peripheral blood mononuclear cells (PBMC) were stained with CD3-AF700 (BD Pharmingen), CD4-PB (BioLegend), CD14-PE-TXRed (Lifetech), CD45RO-FITC (BD Pharmingen), and CD45RA-APC (BioLegend), and the CD3+ CD14− CD4+ CD45RO+ CD45RA− memory T lymphocyte population was sorted by flow cytometry using a Sony SH800Z instrument. RNA was extracted using Total RNA Purification Plus Micro Kit (Norgen Biotek), cDNA synthesized using SuperScript VILO cDNA Synthesis Kit (Invitrogen), and quantitative real-time PCR performed using a custom-made TaqMan Low Density Array (TLDA) (Applied Biosystems) that has been previously described.³ Thermal cycling was performed using a ViiA 7 Real-Time PCR System (Applied Biosystems). A panel of six housekeeping genes was included in the TLDA plates (GAPDH, 18S, ACTB, PPIA, RPLP0, and UBC), of which GAPDH was identified as the most stably expressed among all samples analyzed using the geNorm algorithm.⁴ Raw cycle threshold numbers of amplified gene products were therefore normalized to GAPDH. Cumulative restriction factor expression scores were calculated as the sum expression of all restriction factors of an individual, as previously described.⁵

Total HIV DNA was quantified by digital droplet PCR as previously described.⁶ In brief, genomic DNA was extracted from CD4+ T cells, digested with *Bsa*JI, mixed with primers and probes to detect HIV gag and the housekeeper RPP30, droplets prepared using the QX100 Droplet Generator (BioRad) followed by PCR and then analysis using the QX100 Droplet Reader. Inducible HIV was quantified by quantitative viral outgrowth assay as previously described.⁷ In brief, CD4+ T cells were cultured at limiting dilutions and stimulated with interleukin 2, phytohemagglutinin (PHA), and irradiated allogeneic feeder cells from HIV-uninfected donors in the presence of Molt-4 cells. After 14 days wells were screened for outgrowth of a viral isolate by enzyme-linked immunosorbent assay (ELISA) for p24 (NCI Frederick) to calculate the probable frequency and range of infectious units.

Results and Discussion

Memory CD4+ T cells were sorted by flow cytometry from 21 HIV-1-infected ART-suppressed individuals and 10 uninfected donors on the basis of CD3+ CD4+ CD45RO+ CD45RA− staining (Fig. 1A). RNA was isolated from the sorted cell fraction, cDNA synthesized, and transcriptional restriction factor expression quantified by microarray (Supplementary Table S2). Analysis of total restriction factor

expression between the groups demonstrated a significantly higher average score for cumulative restriction factor expression among ART-suppressed individuals compared with uninfected donors (Fig. 1B) ($p = .036$). Within HIV-infected individuals, cumulative restriction factor score did not significantly correlate with CD4+ T cell counts or CD4/8 cell ratios. It is well-established that ART-suppressed individuals show increased levels of immune activation compared with uninfected individuals, and this likely contributes to much of the increased restriction factor expression we observed.⁸ These results add to previous studies that have found strong upregulation of restriction factors in untreated infection, but no difference between uninfected and ART-suppressed individuals when studying fewer donors and the total CD4+ cell populations.⁵ Discrepancy could also arise from differences in study cohort characteristics or measurement techniques; further studies will be necessary to validate the altered expression of restriction factors between uninfected and ART-suppressed individuals.

Each restriction factor was independently tested for differential expression between ART-suppressed and healthy individuals (Fig. 1C). Expression of eight restriction factors differed significantly and with a false discovery rate of <10%. Of these, five were upregulated in HIV-1-infected individuals: APOBEC3G ($p = .004$), ISG15 ($p = .006$), LGALS3BP ($p = .007$), RNASEL ($p = .007$), and MX2 ($p = .008$). Three restriction factors showed decreased expression in HIV-1-infected individuals: CDKN1A ($p = .01$), TRIM11 ($p = .01$), and BRD4 ($p = .01$). The decreased expression of these restriction factors is unlikely to be a consequence of the increased immune activation in HIV-1-infected individuals because of the opposing effect on expression induced by interferon and in viremic HIV-1 infection.

The restriction factor with the largest magnitude of downregulation observed was CDKN1A (p21) (Fig. 1D). CDKN1A restricts HIV-1 transcription by inhibiting cyclin-dependent kinase-mediated phosphorylation of viral reverse transcriptase, blocking synthesis of cellular dNTPs, and phosphorylating expression of SAMHD1.⁹ Associations were examined between CDKN1A expression and HIV-1 reservoir size, within the 21 infected individuals. No significant association was found with measurements of HIV-1 DNA (Fig. 1E) or inducible virus (Fig. 1F). When accounting for multiple testing we observed no association between the expression of any of the 42 restriction factors measured, and either of these measurements of reservoir size (Supplementary Table S3). A caveat is that reservoir measurements were made using total CD4+ cells, but these results are consistent with a previous study of total CD4+ cells from ART-suppressed individuals that found CDKN1A expression associated with cell associated HIV-1 RNA but not measurements of HIV-1 DNA, and did not test for association with replication-competent inducible virus.²

In contrast to the general observation of increased restriction factor expression, likely as a consequence of the increased immune activation in HIV-1-infected individuals, decreased expression was observed in virally suppressed compared with uninfected individuals for three specific restriction factors: CDKN1A, TRIM11, and BRD4. This may result from decreased expression of certain restriction factors in response to cell stimuli or could indicate changes in the composition of the CD4+ memory T cell population in HIV-

FIG. 1. Host restriction factor expression by memory CD4+ T cells from HIV-1-infected individuals with effective ART. Representative flow cytometry sorting of memory CD4+ T cells (**A**). Cumulative restriction factor RNA copy number compared between healthy donors ($n=10$) and ART-treated virally suppressed HIV+ individuals ($n=21$) (**B**). Mean expression of each restriction factor in ART-suppressed individuals relative to healthy donors. Statistical comparisons are by unpaired t -test (* $p < .05$, ** $p < .01$, not corrected for multiple testing) (**C**). CDKN1A (p21) expression shown for each healthy donor and HIV+ ART-suppressed individual (**D**). CDKN1A expression shown relative to the size of the viral reservoir in infected individuals, quantified by measuring HIV-1 DNA (**E**) or replication-competent inducible HIV-1 virus (**F**). Statistical analyses were performed by two-tailed Pearson's correlation. ART, antiretroviral therapy.

1-infected individuals with suppressed viral load. Memory T cell subsets likely differ in their intrinsic expression levels of restriction factors, and ART-suppressed individuals may exhibit an overrepresentation of a memory T cell subset with lower expression of these restriction factors. This perturbation could occur as a result of viral infection, immune response to infection, or immune reconstitution after ART. Determining the basis of decreased CDKN1A, TRIM11, and BRD4 expression in virally suppressed individuals could identify changes in the memory T cell compartment with significance for the persistence of HIV-1 during ART. We hope this initial characterization of *in vivo* changes in primary human samples will lead others to explore the basis of distinct restriction factor expression in virally suppressed individuals.

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Author Disclosure Statement

No competing financial interests exist.

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