AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 31, Number 7, 2015

DOI: 10.1089/aid.2014.0297

Short Communication: Expression of APOBEC3G and Interferon Gamma in Pleural Fluid Mononuclear Cells from HIV/TB Dual Infected Subjects

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Abstract

Sites of HIV/TB coinfection are characterized by increased HIV-1 replication and a TH1 profile. However, expression of HIV-1 restriction factors, such as APOBEC3G (A3G) *in situ*, is unknown. Using an RT-profiler focused on genes related to HIV-1 expansion, we examined pleural fluid mononuclear cells (PFMCs) from patients with HIV/TB coinfection in comparison to HIV-uninfected patients with TB disease. Significant expression of interferon (IFN)-γ and restriction factors A3G and A3F and TRIM5α in PFMCs was found. Genes correlating significantly with the expression of IFN-γ included A3G and A3F. However, pleural fluid HIV-1 viral load and HIV-1 *gag/pol* mRNA in PFMCs did not correlate with A3G activity.

Pleural sites of *Mycobacterium tuberculosis* (MTB) infection are characterized by excessive TH1 cytokine expression. Interferon gamma (IFN-γ) is one of four cytokines highly present in pleural fluid from TB patients regardless of HIV-1 coinfection.[1] IFN-γ-induced polarization of naive human CD4 T cells into TH1 cells *in vitro* supports the expression of HIV-1 restriction factors, APOBEC3G (A3G) and −3F (A3F).[2] A3G leads to defective HIV-1 virus production through deamination of cytidine to uridine in the negative strand of HIV-1 RNA.[3] This is inhibited by the HIV-1 accessory protein vif through promotion of ubiquitination and thereby degradation of A3G.[3] However, A3G also inhibits HIV-1 reverse transcription through a nonediting/vif-independent modality.[4] Recently, both A3G's deaminase activity and its nonediting function were found to contribute to restriction of wild-type HIV-1 replication in infected subjects.[5] A3G has been reported in two bioforms. The first is a low-molecular-mass (LMM) form, which packages into virions and restricts HIV-1 replication in newly infected mononuclear cells.[6] LMM is present in cell types poorly permissive to HIV-1 replication, such as resting CD4 T cells, monocytes, and dendritic cells.[7] A second form of A3G is a high-molecular-mass (HMM) complex, which contains one or more inhibitory RNAs and has no enzymatic activity.[8] HMM A3G is present in CD4 T cells activated by TCR stimulation or by cytokines.[9]

To assess the expression of genes involved in the HIV-1 life cycle and HIV-1 restriction factors at sites of HIV/TB dual infection, we examined gene expression of pleural fluid mononuclear cells (PFMCs) from patients with tuberculosis (TB) disease and HIV/TB coinfection. Symptomatic patients with fever, cough, night sweats, and dyspnea for at least 2 weeks and chest X-ray evidence of moderate to large pleural effusions were recruited at Mulago Hospital (Kampala, Uganda). These subjects were referred to the Tuberculosis Clinic for the evaluation of pleural TB as previously described.[1] Written informed consent approved by the institutional review boards at Makerere University (Kampala, Uganda) and Case Western Reserve University/University Hospitals of Cleveland (Cleveland, OH) was obtained from all subjects. All subjects were started on TB treatment after diagnostic work-up for pleural TB disease was completed. The diagnosis of TB disease was confirmed by positive culture for MTB in pleural fluid and/or pleural biopsy and/or sputum. A total of 14 HIV/TB-coinfected patients and four subjects with HIV-uninfected TB disease were enrolled. HIV/TB-coinfected and TB disease patients were comparable in age (average 33 years old) and sex (two-thirds were male). None of the HIV/TB-coinfected patients was on antiretroviral treatment at the time of the study. HIV/TB-coinfected subjects with pleural TB disease had a median CD4 T cell count of 169 (range: 59–445) and plasma viral

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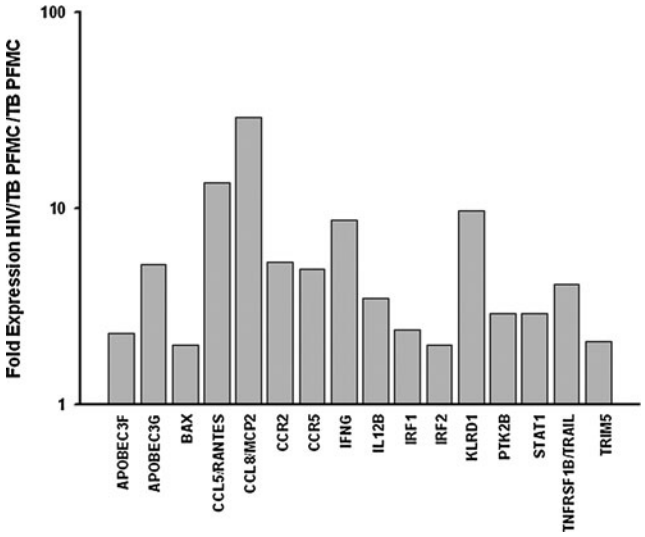

FIG. 1. The profile of HIV-1-related genes in pleural fluid mononuclear cells (PFMC) from subjects with HIV/tuberculosis (TB) coinfection and TB disease. Total RNA from PFMC of four HIV/TB-coinfected patients and four subjects with TB disease was isolated and assessed by the reverse transcriptase polymerase chain reaction (RT-PCR) profiler focused on identification of human HIV-1 infection and host response genes. The fold difference between HIV/TB-coinfected patients and patients with TB disease for each gene was calculated as the average of expression in subjects with HIV/TB coinfection over an average of expression in subjects with TB disease using the statistical package provided with the RT-profiler (SA Biosciences). Genes with a difference in expression in HIV-1-infected subjects by ≥2-fold and $p < 0.05$ are shown.

load of 2.8×10^5 (range: 0.2–9.8×10^5). Assessment of CD4 counts was not performed on HIV-uninfected subjects with pleural TB disease in this study.

PFMCs freshly prepared from pleural fluid were assessed by immunostaining and FACS analysis on site in Uganda. PFMCs contained 40–60% CD4 T cells and 1–5% $CD14^+$ macrophages Additional aliquots of PFMCs were suspended in RNeasy buffer (Qiagen/SA Biosciences, Valencia, CA) at 2×10^6/vial or Tri-reagent (Invitrogen, Carlsbad, CA) at 10×10^6/vial and samples were stored frozen until shipment to Case Western Reserve University for further processing.

RNA was isolated from frozen samples and assessed for gene expression by polymerase chain reaction (PCR) Array using RT^2 Profiler [Human HIV Infection and Host Response (PAHS-051A)] (SA Biosciences/Qiagen) according to the manufacturer's instructions. This assay assesses the expression of 84 host genes involved in the HIV-1 life cycle, including viral entry, integration, and gene transcription. Real-time PCR was performed using OneStep Real Time PCR machine (Applied Biosystems/Lifetechnologies, Foster City, CA). Relative gene expression was calculated by the ΔC_t method. In this assay, expression of each gene is corrected with that of the housekeeping gene, GAPDH, included in the assay. Fold expression for each gene (the average of the expression in four subjects with HIV/TB dual infection divided by that in four subjects with TB monoinfection) was calculated employing the statistical package provided (SA Biosciences / Qiagen). This package uses ANOVA with correction for multiple comparisons.

Differences (fold ≥ 2) in gene expression between HIV/TB-coinfected subjects and TB subjects were found in 30 of 84 genes. Of these, all but two genes (CD4 and CXCL12) were upregulated in PFMCs from HIV/TB-coinfected subjects. Of all genes (induced or suppressed) in HIV-1-coinfected subjects, however, only 16 genes were significantly different ($p < 0.05$) (Fig. 1). Higher expression of IFN-γ and genes involved in either regulation of its expression (IL-12β) or signaling (IRF1, IRF2, and STAT1) was found. Expression of HIV-1 restriction factors A3G, A3F, and TRIM5α was also significantly higher in PFMCs from HIV/TB-coinfected subjects. Other significantly upregulated genes included molecules involved in viral entry: CCL5 (RANTES), CCL8 (MCP-2), the chemokine receptors CCR5 and CCR2, and the apoptosis molecules Bax and TNFSF10 (TRAIL). Of interest, there was a significantly higher expression of KLRD1

Table 1. Genes Involved in the HIV-1 Life Cycle that Correlate with the Expression of Interferon-γ

	Correlation	*Significance* p *value*	*Known function in HIV-1 infection*	*Reference*
APOBEC3G	0.82	< 0.01	HIV-1 restriction factor family of DNA cytosine deaminases	2
APOBEC3F	0.84	< 0.001	HIV-1 restriction factor family of DNA cytosine deaminases	2
Bcl11B (CTIP-2)	0.76	< 0.01	Transcriptional repressor of HIV-1 LTR in T cells	1, 2
CCL5 (RANTES)	0.81	< 0.01	Ligand of CCR5. Critical to the recruitment of CCR5 expressing cells. Inhibits HIV-1 entry/replication	14
CCL8 (MCP-2)	0.68	< 0.02	Ligand of CCR5. Inhibits HIV-1 entry/replication and potentiates the effect of RANTES	11
CCR2	0.62	< 0.05	Receptor for MCP-1. MCP-1/CCR2 interaction is involved in the recruitment of target cells, increasing CXCR4 expression and Th2 biased immune response	15
CCR5	0.78	< 0.01	Receptor for RANTES and MCP-2. Coreceptor of HIV-1, indispensable in CCR5 tropic HIV-1 entry	16

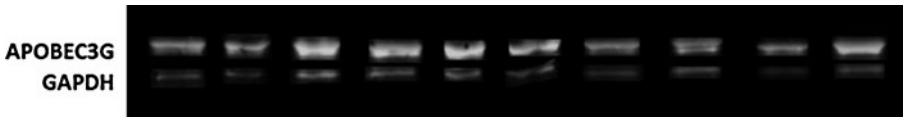

FIG. 2. Expression of APOBEC3G in PFMC from HIV/TB-coinfected subjects. Western blot of protein purified from PFMC from HIV/TB-coinfected subjects ($n=10$) was assessed for APOBEC3G and GAPDH. After quantification of bands by densitometry, APOBEC3G was corrected to GAPDH for each subject.

(CD94), previously found to be expressed on activated CD8 T cells in PBMCs of viremic HIV-1-infected subjects. More recently, KLRD1 (CD94) (and NK2GA with which it heterodimerizes) was found to be highly expressed by TH1 CD4 T cells in a model of influenza virus infection.[10]

To understand the relationship between molecules identified by the RT-PCR profiler and IFN-γ expression, we correlated the expression of IFN-γ and the expression of all individual host PFMC genes identified by the RT-Profiler from all subjects regardless of HIV-1 infection. The correlation between genes was assessed by Spearman rank analysis. A p value of <0.05 was considered significant. We found a significant correlation ($R=0.6$ and $p<0.05$) between the expression of IFN-γ and a select group of 16 genes. Of these genes, seven were involved in the HIV-1 life cycle (Table 1). A strong relationship between the expression of IFN-γ and both HIV-1 restriction factors A3G and A3F (but not Trim 5α) was found. IFN-γ expression significantly correlated with the expression of beta chemokines (CCL5 and CCL8) and their receptor (CCR5). Of note, CCL8 synergizes with CCL5 in the inhibition of HIV-1 entry through CCR5.[11] High expression of both these HIV-1-inhibiting chemokines (Fig. 1) and CCR5, and their correlation with IFN-γ *in situ*, indicates a possible dual role for IFN-γ in the spread of CCR5 tropic HIV-1. Specifically, its effect on CCL8 and CCL5 expression may serve to dampen its effects in promoting HIV-1 infection through upregulation of CCR5. Another gene significantly correlating with IFN-γ was BCL11B, a bifunctional transcription factor with exclusive expression in T cells and critical to the maintenance of T cell development. BCL11B, both directly and indirectly, silences HIV-LTR.[12] In a recent study, BCL11B and the NuRD complex initiated silencing of TAT-activated HIV-LTR.[12] Conversely, through promotion of TCR/CD28-mediated NF-κB activation, BCL11B maintains immune activation *in situ*.[13] It is intriguing to postulate that at pleural sites of TB characterized by high IFN-γ activity, requirements for the establishment of latent HIV-1 infection are being met simultaneously with the sustenance of TH1 immune responses and the promotion of HIV-1 infection through immune activation. These features of the molecular profile of HIV-1 infection need to be further investigated.

To evaluate the role of A3G in HIV activity in PFMCs, in a larger ($n=10$) group of HIV/TB-coinfected patients, PFMCs were assessed for A3G and HIV-1 *gag/pol* mRNA and the housekeeping gene GAPDH by RT-PCR as previously reported,[1] and A3G protein by Western blot (see Supplementary Methods; Supplementary Data are available online at www.liebertpub.com/aid). We confirmed the presence of A3G mRNA and protein in PFMCs from all subjects. A3G mRNA correlated with IFN-γ mRNA ($R=0.6$, $p<0.02$) and was present by Western blot (Fig. 2). However, we did not find a correlation between A3G mRNA or protein and either HIV-1 viral load in pleural fluid or HIV-1 *gag/pol* mRNA in PFMCs from HIV/TB subjects. Lack of a negative correlation between A3G and HIV-1 transcriptional activity in pleural fluid or PFMCs from HIV/TB-coinfected patients implies that cellular A3G may be biologically ''inactive'' and may reflect that at sites of immune activation such as TB, A3G may be present in an HMM form with no HIV-1 restriction capacity. This interpretation is consistent with low levels of biologically active A3G in *in vitro*-activated as compared to resting mononuclear cells.[9] It is also possible that any HIV-1 *vif* activity on the degradation of A3G *in situ* is limited to LMM A3G or is inadequate for HMM A3G. These considerations need to be studied further.

Acknowledgments

This study was supported by NIH Grants HL-051636 and AI-1080313, Center for AIDS Research Grant P30 AI-036219, and the Tuberculosis Research Unit Grant AI-70022.

Author Disclosure Statement

No competing financial interests exist.

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