Short Communication

Induction of HIV Type 1 Expression Correlates with T Cell Responsiveness to Mycobacteria in Patients Coinfected with HIV Type 1 and *Mycobacterium tuberculosis*

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Abstract

An *in vitro* mononuclear cell system to model the microenvironment of coinfection with HIV-1 and *Mycobacterium tuberculosis* (MTB) was developed. This cellular system was used to assess the interaction of MTB-infected monocytes and T cells from dually infected HIV-1/TB patients with pulmonary tuberculosis (TB). Subjects with higher induction of HIV-1 gag/pol mRNA expression after MTB stimulation had increased MTB-specific T cell IFN- γ and TNF- α production. Lack of HIV-1 mRNA induction did not correlate with increased induction of regulatory T cells (T-reg) as measured by MTB-induced Foxp3 mRNA. HIV-1 induction did not significantly correlate with clinical parameters including plasma HIV-1 viral load or CD4⁺ T cell count. These data model MTB-induced HIV-1 replication at the microenvironment of MTB reactivation/infection. The data suggest that the magnitude of MTB-specific T cell responses drives local viral pathogenesis regardless of the stage of HIV-1 disease as reflected by plasma viral load or CD4⁺ T cell count.

IN UNDERDEVELOPED COUNTRIES, tuberculosis (TB) is one of the most common causes of morbidity and the most common cause of death in HIV-1-infected adults.¹ Both increased progression of HIV-1 disease, and mortality from HIV-1 infection have been reported in dually infected HIV/TB subjects.² To date several mechanisms of increased HIV activity during TB have been documented at sites of *Mycobacterium tuberculosis* (MTB) infection. These include both transcriptional activation of HIV-1-infected cells to viral production and new rounds of infection of uninfected cells.³

Transcriptional activation of HIV-1 by MTB and cytokines associated with active TB, such as TNF- α , has previously been reported.^{4,5} However, the cellular basis for induction of viral transcription from HIV-1-infected mononuclear cells remains unclear. It has been shown that MTB-infected mononuclear phagocytes may transactivate HIV-1 in cells latently infected with HIV-1, and that this may serve a role in increased HIV-1 activity *in vivo*.⁶ This scenario may be particularly prominent

at sites of MTB infection, and affect viral dynamics prior to systemic immune activation due to active TB disease.⁷

In an *in vitro* study from 1996, Golletti *et al.* showed that increased HIV-1 replication occurs in T cells from latently MTB-infected individuals who were HIV-1 infected upon stimulation by purified protein derivative (PPD) of MTB.⁸ The current study extends these findings using the more recently developed sensitive and specific quantitative real-time PCR methodology. Here, mononuclear cells from HIV-1-infected patients with active pulmonary TB were employed. A cellular system of low frequency of MTB infection that models the low bacillary burden of sites of MTB infection was used.⁶ This low bacillary burden best reflects the microenvironment of MTB infection at initiation of reactivation TB or during primary progressive TB.

HIV-1-seropositive patients with newly diagnosed pulmonary TB were identified at the National Tuberculosis Treatment Centre, Kampala, Uganda between July 2004 and

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January 2008. A diagnosis of TB was confirmed by positive culture of sputum. None of the subjects had received anti-retroviral or TB therapy prior to this study. The clinical and laboratory characteristics of the subjects are presented in Table 1. The age range was 18–48 years old. Of the subjects 11/18 were females. All but one subject had grade 2 or 3 (i.e., cavitory TB) disease at the time of diagnosis.

Low dose MTB-infected monocytes (MN) were incubated with autologous nonadherent cells (NACs), and HIV-1 induction was determined by measuring the expression of HIV-1 late gene product gag/pol mRNA after 5 days. Input HIV-1 (in NAC) was assessed by HIV-1 ssDNA in peripheral blood mononuclear cells (PBMCs) at time zero as before.⁹

Adherent MN were purified from PBMCs then rested overnight. The purity of adherent MN was found to be 95% by Wright staining. Avirulent MTB (H37Ra) at 1:1 (bacteria: cell) in 30% v/v unheated pooled human serum was added to the replicate wells for 90 min and then washed extensively. By AFB staining an average of 1–3% of MN were infected. Autologous NACs were then returned to MTB-infected and -uninfected MN at a ratio of 10:1 (NAC:MN). Cultures were maintained for 120 h. Then, NACs were harvested, and supernatants and cell lysates (Tri-reagent from Mol Research, Cincinnati, OH) were prepared. Total cellular RNA was isolated from cell lysates as before.⁹ RNA was assessed for HIV-1 gag/pol expression by real-time RT-PCR (Taqman). As HIV-1 clade A/D predominates in Uganda,^{10,11} a specific assay was developed and validated. Probe: 6FAM-CCA GGA AAA TGG AAA CC- MGBNFQ; Forward: AAG CTC TAT TAG ATA CAG GAG CAG ATG ATA; Reverse: CAA TTA TGT TGA CAG GTG TAG GTC CTA. Reagents for ribosomal 18S expression were as before.⁹ Quantities of mRNA were determined by using a dilution series of cloned target cDNA in each assay. In each sample, mRNA copies were normalized to the copy number of R18 (1×10^{10} copies of R18 in 10^6 cells). Fold HIV-1 induction was calculated as follows: copies of gag/pol in (MTB-infected culture/medium alone).

TABLE 1. CLINICAL AND LABORATORY PARAMETERS

Subject	HIV-1 induction	Plasma HIV-1 VL	CD4	Extent of TB Dz ^a
1	0.14	1833	222	3
2	0.59	14814	302	3
3	0.64	209,000	448	1
4	0.72	180,982	253	2
5	0.72	160,000	287	3
6	1.0	440,000	200	3
7	2.1	47,591	239	3
8	3.4	7811	376	2
9	3.4	86,500	271	2
10	3.7	423,000	352	3
11	3.9	242,000	74	3
12	5.4	182,000	255	3
13	6.5	437,000	189	3
14	7.2	56,000	133	2
15	7.8	24,000	493	3
16	8.4	121,000	201	3
17	11.4	851,000	219	3
18	36.0	980,000	244	3
Median	3.6	170,491	249	

^aExtent of disease: 1, limited unilateral noncavitary; 2, unilateral with cavitation; 3, extensive bilateral with cavitation.

There was significant heterogeneity in the amount of MTB-induced HIV-1 induction. HIV-1 induction ranged from 0.14- to 36-fold with a median induction of 3.6-fold (Table 1). Of note, the input HIV-1 assessed by HIV-1 ssDNA did not correlate with the level of HIV mRNA induction, suggesting that the amount of HIV-1 present at the time of MTB stimulation did not affect the degree of HIV-1 induction observed at 120 h (data not shown).

To determine if the significant heterogeneity in induction of HIV-1 was related to the degree of MTB-specific T cell activation, cytokines [interferon-gamma (IFN- γ) and tumor necrosis-alpha (TNF- α)] in culture supernatants were assessed using commercial ELISA kits. There was a significant correlation between both MTB-induced cytokines in culture supernatants at 5 days and the induction of HIV-1. Using Spearman analysis, Fig. 1A demonstrates the correlation ($r = 0.55$, $p = 0.019$) between HIV induction and IFN- γ levels. Figure 1B demonstrates a correlation ($r = 0.76$, $p = 0.0003$) between HIV-1 induction and TNF- α levels. Use of NACs (rather than CD8-depleted CD4⁺ T cells) allows for the cytokines in cultures to originate from CD4⁺ T cells as well as CD8⁺ T cells. Presumably, any CD8⁺ T cells would also possess cytotoxic T lymphocyte (CTL) activity. Since we were able to detect significant correlations between HIV induction and IFN- γ and TNF- α levels, this implies that the cytokines are coming predominantly from CD4⁺ T cells that are capable of HIV induction rather than activated CD8⁺ CTL that would blunt HIV induction by lysing the HIV-infected CD4⁺ T cells.

FIG. 1. Correlation of HIV-1 induction and IFN- γ and TNF- α . Low dose MTB-infected MN incubated for 5 days with autologous NAC in HIV/TB patients with active TB. Induction of HIV-1 gag/pol mRNA correlated with IFN- γ (A) and TNF- α (B) in the supernatant. Cytokine levels and HIV induction are presented in log scale.

Correlations between the clinical data and MTB-induced HIV-1 induction were also examined. There was only a trend but no significant correlation between MTB-induced HIV-1 mRNA and plasma viral load ($r = 0.43$, $p = 0.075$), indicating that T cell responses *in situ* do not correlate strongly with systemic viral dynamics. Further, no correlation between MTB-induced HIV-1 mRNA and CD4 count ($r = -0.30$, $p = 0.23$) or age ($r = 0.35$, $p = 0.16$) was found.

Our study found a number of individuals whose T cells did not respond vigorously to MTB. However, reduced MTB-specific T cell cytokine responses did not correlate with a lower total CD4⁺ T cell count ($r < 0.20$, $p > 0.50$ for both IFN- γ and TNF- α). The expansion of regulatory T cells (T-reg) has been documented during TB,¹² and dual HIV/TB infection (Z. Toossi and C.S. Hirsch, unpublished observations). Previously, control of HIV-1 viremia was found to correlate with degree of T-reg expansion.¹³ We examined mRNA levels of the T-reg marker, Foxp3, in a subset of 12 individuals using Taqman primers and probe for ribosomal 18S RNA and Foxp3 as previously described.^{6,14} We found no correlation between levels of Foxp3 mRNA and MTB-induced HIV-1 expression ($r = -0.06$, $p = 0.86$), suggesting that T-reg was not responsible for the differences in HIV-1 mRNA induction observed.

Previously, a modest although statistically significant increase in plasma HIV-1 viral load in individuals with active TB as compared to individuals without active TB matched for CD4 T cell count has been shown.¹⁵ The basis for this excess viral activity has been related to both new rounds of HIV infection and transcriptional activation at sites of TB.¹⁶ This study demonstrates that induction of HIV-1 transcription in mononuclear cells from HIV-1-infected patients with pulmonary TB correlates with increased MTB-specific T cell responses. TCR-induced activation of T cells is well known to increase HIV replication. IFN- γ and TNF- α produced by these activated cells may feedback on the MTB-specific CD4⁺ T cells and bystander CD4⁺ T cells to account for a portion of the HIV induction.¹⁷

Our *in vitro* culture system was developed to model the microenvironment of sites of MTB infection with low bacillary burdens, such as the lung at times of reactivation of TB, pleural TB, or draining lymph nodes in active pulmonary TB patients. Significant cellular interactions *in vivo* leading to induction of HIV-1 replication are likely at these sites that may affect ensuing viral dynamics. Interestingly, HIV induction in our *in vitro* cell model showed only a trend toward a very modest correlation with systemic viral load ($r = 0.43$, $p = 0.075$). However, at the time of diagnosis of TB, plasma viral load likely reflects systemic immune activation, more so than events of the coinfection *in situ*.⁷

Our studies further suggest that the microenvironment of dual infection is important to the pathogenesis of both diseases. Our data show that increased MTB-specific T cell responses can drive significantly higher amounts of local HIV replication. This scenario is particularly likely at times of low MTB infection such as the setting of reactivation of TB. Enhancement of HIV-1 replication *in situ* at these early time points thus may overwhelm the immune system to promote a more rapid course of HIV disease. Under this scenario, promotion of HIV disease by MTB is initiated at times of reactivation of TB and may extend well into treatment.¹⁸ Uncontrolled HIV-1 infection, in turn, undermines MTB immune responses. Our data provide mechanistic support for

this observation and support treating both MTB and HIV in dually infected individuals to arrest the cycle of TB-induced HIV-1 pathogenesis.

Acknowledgments

This work is supported by Grants AI-73217, HL-51636, AI-36219, and AI-95383.

Disclosure Statement

No competing financial interests exist.

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