CONCISE COMMUNICATION

Activation of β-Chemokines and CCR5 in Persons Infected with Human Immunodeficiency Virus Type 1 and Tuberculosis

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Tuberculosis (TB) in human immunodeficiency virus type 1 (HIV-1)–infected persons is associated with progression of HIV-1 disease. The expression of macrophage inflammatory protein (MIP)–1α and CCR5 was assessed in HIV-1–infected patients with pulmonary TB (HIV-1/PTB) and without PTB (HIV-1/C), PTB patients not infected with HIV-1 (PTB), and control subjects. *Mycobacterium tuberculosis* (MTB)–induced MIP-1α production was lower in peripheral blood mononuclear cells (PBMC) of HIV-1/PTB patients than in those of PTB patients ($P < .05$) and was lower in PBMC of HIV-1/C patients than in those of control subjects ($P < .005$). However, MIP-1α production was higher in PBMC of HIV/PTB patients than in those of HIV-1/C patients ($P < .01$). The pattern of MTB-induced RANTES production was similar to that of MIP-1α. However, MTB induced greater expression of mRNA for CCR5 in PBMC of HIV-1/PTB patients than in those of HIV-1/C patients ($P < .04$). Furthermore, the MTB-induced HIV p24 antigen level in PBMC of HIV-1/PTB patients with a CD4 cell count <500 cells/μL was higher ($P < .05$) than that in HIV-1/C patients. Thus, perturbations in chemokine pathways in HIV-1/PTB patients may accelerate HIV-1 disease.

Tuberculosis (TB) occurs during all stages of human immunodeficiency virus type 1 (HIV-1) infection [1, 2] and adversely affects the course of HIV-1 infection [3, 4]. The mechanisms by which TB leads to augmentation of HIV-1 load and activity are not fully understood. Active TB leads to enhanced production of mononuclear phagocyte cytokines, such as interleukin (IL)–1β, tumor necrosis factor (TNF)–α, and IL-6 [5], which activate HIV-1 replication [6]. *Mycobacterium tuberculosis* (MTB) and its components increase HIV-1 replication in mononuclear cells that are latently or acutely infected with HIV-1 [7, 8]. In addition, peripheral blood monocytes from patients with active TB are more susceptible to HIV-1 infection in vitro than are monocytes from healthy subjects [9]. However, it is not known whether active TB enhances the HIV infectivity of target cells.

The successful entry of HIV-1 into target cells requires the chemokine receptor, CCR5 [10], plus CD4 molecules. The natural ligands for CCR5 are the β-chemokines, namely, macrophage inflammatory protein (MIP)–1α and –1β and RANTES. β-chemokines are produced by CD8 and CD4 lymphocytes and by mononuclear phagocytes [11]. β-chemokines may have a dual role in HIV-1 infection. By binding to CCR5, they may inhibit HIV-1 infection of target cells [12] but also may induce HIV-1 replication in infected mononuclear cells [13]. In this study, we assessed the expression of MIP-1α and CCR5 in patients with active pulmonary TB (PTB) with (HIV-1/PTB) and without (PTB) HIV-1 infection and investigated whether dysregulations in β-chemokines and CCR5 were associated with higher HIV-1 activity in HIV-1/PTB patients.

Methods

Study population. HIV-1/PTB and PTB patients were recruited from the National Tuberculosis Control Programme clinic at Mulago Hospital in Kampala, Uganda. Eligibility criteria for patient enrollment were as noted in [14], which accompanies this report. In total, 67 HIV-1/PTB and 57 PTB patients were enrolled. Control subjects (C) were chest and medical clinic patients and healthy volunteers ($n = 25$). HIV-1–infected patients without PTB (HIV-1/C; $n = 39$) were recruited from the AIDS support clinic at Mulago Hospital. HIV-1/C patients had ⩾1 other cocondition and a mean CD4 cell count of 409 ± 257 cells/μL. Active PTB was ex-

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Informed consent was obtained from all study participants. The study protocol was reviewed and approved by the institutional review boards at Case Western Reserve University and the Ugandan National AIDS Research Subcommittee.

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cluded in HIV-1/C patients and control subjects by physical examination and by normal chest radiograph. Blood (25–30 mL) was collected from each subject into Vacutainer tubes containing heparin, EDTA, or citrate.

Cell culture. Peripheral blood mononuclear cells (PBMC) were isolated, as described elsewhere [15], and were resuspended at 3×10^6/mL in RPMI 1640 (Bio-Whittaker) supplemented with L-glutamate (2 μM), penicillin and streptomycin (1 U/mL and 100 μg/mL, respectively), 20 mM HEPES, and 2% heat-inactivated pooled human serum. PBMC received an avirulent strain of MTB, H37Ra, or medium alone. Aliquots of log phase cultures of H37Ra containing 2×10^8 cfu/mL were prepared. For stimulation, an aliquot of MTB was vortexed with glass beads for 15 min and then was added to PBMC at an infection ratio of 10:3 MTB bacteria: cell. Cell pellets (in Tri-reagent; Molecular Research Center) and culture supernatants were harvested at 3, 6, 24, and 120 h.

ELISA for β-chemokines and HIV-1 p24. MIP-1α and RANTES immunoreactivities were quantitated by commercial ELISA kits (R&D Systems) with limits of detection of 6.0 and 5.0 pg/mL, respectively. HIV-1 p24 was measured by kinetic ELISA (Coulter Immunology), which has a lower sensitivity of 7.5 pg/mL.

Measurement of mRNA expression. Expression of MIP-1α and CCR5 in total RNA was assessed by the RiboQuant Multi-Probe RNase protection assay (RPA; PharMingen), according to the manufacturer's instructions. In brief, total RNA (2 μg) was hybridized overnight to a ^{32}P-labeled RNA probe cocktail containing CCR5, MIP-1α, and 2 housekeeping genes *(L32* and *GAPDH).* The samples then were incubated with RNase A, to digest unhybridized RNA. Digests were run on a 5% denaturing polyacrylamide gel together with the undigested probe, which served as a marker. The gel was dried and was exposed to Kodak film at −70°C. We determined the densitometry of the autoradiographs, to assess the intensity of the bands.

Flow cytometry. Surface expression of CCR5 on mononuclear cells in whole blood was assessed by immunostaining and flow cytometry. In brief, 100 μL of blood was incubated with combinations of fluorochrome-conjugated antibodies to CD3 (fluorescein isothiocyanate; Becton Dickinson), CD4 (RPE-Cy5; Dako), and human CCR5 (phycoerythrin [PE]; R&D Systems) or IgG2a isotype control antibody (PE; R&D Systems) at 4°C for 30 min. Red blood cells were lysed, and the cells were washed 3 times in PBS with 1% bovine serum albumin and then were fixed in 250 μL of paraformaldehyde. Samples were acquired by FACScan flow cytometer (Becton Dickinson). Data were analyzed with WinMDI software (Scripps Institute).

Statistical analysis. Data were analyzed with an SAS statistical package. We used analysis of variance (ANOVA) and Student's t test to test differences between study groups. $P < .05$ was considered to be significant. Linear regression and correlation analyses were used to correlate findings within groups.

Results

MTB-induced β-chemokines. First, we assessed the induction of MIP-1α by MTB in PBMC from the 4 study populations: HIV-1/PTB, HIV-1/C, and PTB patients and control subjects. In preliminary experiments, the in vitro kinetics of induction of MIP-1α by MTB were determined in each group. A rapid upregulation of MIP-1α production in all groups was observed at 3–6 h. Levels peaked at 24 h and plateaued thereafter (data not shown). Next, the cumulative MTB-induced MIP-1α production at 24 h for each study group was assessed (figure 1*A*). The mean levels of MIP-1α among the 4 groups were statistically different ($P = .001$, ANOVA). Analysis of results by linear regression showed that HIV infection had a limiting effect on MTB-induced MIP-1α production: MIP-1α levels were lower in HIV-1/PTB than in PTB patients ($P < .05$) and were lower in HIV-1/C patients than in control subjects ($P < .005$). Only in HIV-1–infected patients with PTB was an enhancing effect on MIP-1α production seen: MTB-induced MIP-1α levels were higher in HIV-1/PTB than in HIV-1/C patients ($P < .02$).

The induction of another β-chemokine, RANTES, by MTB in the same supernatants showed a pattern similar to that of MIP-1α and was limited in HIV-1–infected patients (figure 1*B*). Therefore, the characteristics of MTB-induced β-chemokine production are similar in these 2 chemokines.

Expression of MIP-1α and CCR5 mRNA in PBMC of HIV-

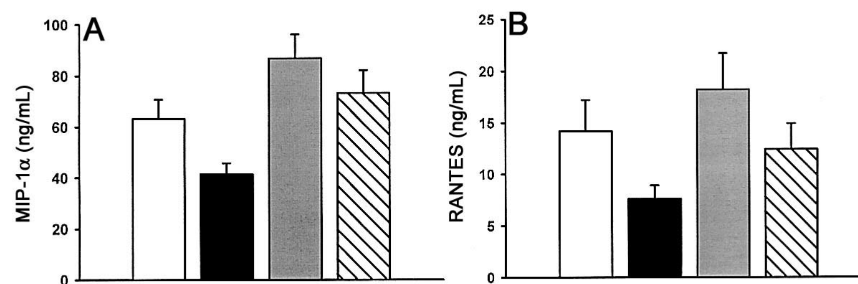

Figure 1. *Mycobacterium tuberculosis* (MTB)–induced macrophage inflammatory protein (MIP)–1α and RANTES production. Peripheral blood mononuclear cells (3×10^6) from persons infected with human immununodeficiency virus type 1 (HIV-1) and pulmonary tuberculosis (PTB) *(open bars),* HIV-1 but no PTB *(black bars),* or PTB alone *(gray bars)* and from control subjects *(hatched bars)* were incubated with MTB, at an infection ratio of 10:3 MTB bacteria:cell. Culture supernatants were harvested at 24 h and were assessed by ELISA for MIP-1α *(A)* and RANTES *(B).* Data are mean ± SE of stimulated-unstimulated chemokine level for each group.

1–infected patients with and without PTB. To concurrently assess MTB-induced expression of MIP-1α and CCR5 mRNA in PBMC, RPA was done on total RNA. The probe cocktail contained the housekeeping gene *L32* in addition to MIP-1α and CCR5. MTB-induced expression of MIP-1α and CCR5 mRNA in PBMC was optimal at 3 h. We compared the relative expression of MTB-induced MIP-1α and CCR5 mRNA in PBMC from HIV-1/PTB ($n = 9$) and HIV-1/C patients ($n = 7$) at 3 h. Densitometry was performed on the bands for MIP-1α, CCR5, and the housekeeping gene, *L32.* The ratio of MIP-1α and CCR5 to *L32* was calculated for each patient, and the means were obtained (figure 2). Expression of MIP-1α mRNA was 1.5-fold higher and that of CCR5 was 2-fold higher ($P < .04$) in HIV-1/PTB patients than in HIV-1/C patients. Thus, MTB had a stronger effect on up-regulation of CCR5 than on MIP-1α mRNA expression in PBMC from HIV-1/PTB versus those from HIV-1/C patients.

Expression of CCR5 on PBMC. To assess the expression of CCR5 in mononuclear cell subpopulations, immunostaining and flow cytometry were done on whole blood. Patients with HIV-1/PTB ($n = 15$) had higher expression of CCR5 on CD4 T cells than did HIV-1/C patients ($n = 13$). However, this difference did not reach statistical significance. In contrast, the expression of CCR5 on CD8 cells was higher in HIV-1/C than in HIV-1/PTB patients. This difference also was not statistically significant. The expression of CCR5 on monocytes from both groups was similar.

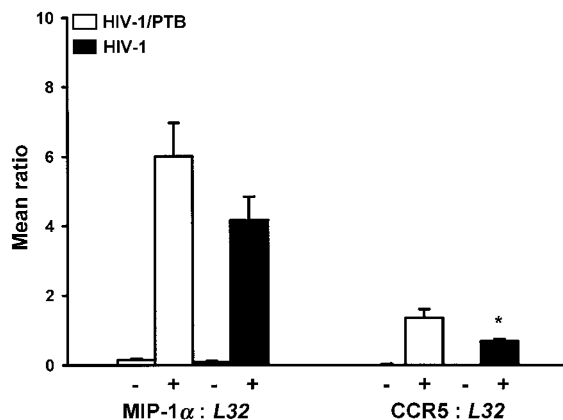

Figure 2. *Mycobacterium tuberculosis* (MTB)–induced expression of macrophage inflammatory protein (MIP)–1α and CCR5. Peripheral blood mononuclear cells (3×10^6) from persons infected with human immunodeficiency virus type 1 (HIV-1) and pulmonary tuberculosis (PTB; $n = 9$) or with HIV-1 alone ($n = 7$) were incubated with MTB, at an infection ratio of 10:3 MTB bacteria:cell (+), or with medium (−). Total RNA was obtained from cell pellets at 3 h and was assessed for MIP-1α and CCR5 by RNase protection assay (PharMingen). Densitometry was performed on bands for MIP-1α, CCR5, and the housekeeping gene, *L32,* and a mean ratio (±SE) of MIP-1α and CCR5 to *L32* was calculated.

Correlation of MIP-1α and HIV-1 load and activity. Next, we analyzed the relationship between MIP-1α production and MTB-induced HIV p24 in 2 CD4 cell–matched groups of HIV-1/PTB and HIV-1/C patients: persons with high (>500 cells/μL) and low (⩽500 cells/μL) CD4 cell counts. There was no correlation between induction of HIV p24 antigen by MTB in PBMC culture supernatants (at 120 h) and MTB-stimulated production of MIP-1α (at 24 h). However, significant induction of HIV p24 antigen was seen only in HIV-1/PTB patients with low CD4 cell counts when compared with CD4-matched HIV-1/C patients ($P < .05$; data not shown). Levels of MTB-induced MIP-1α also were significantly higher in HIV-1/PTB patients than in the HIV-1/C group with low CD4 cell counts ($P < .04$).

Discussion

In this study, we examined the regulation of MIP-1α and its receptor CCR5 by MTB and during active PTB in patients with HIV-1 infection. Findings were compared with those of MTB-sensitized HIV-1–infected patients in whom TB was excluded but who had ⩾1 other cocondition. CCR5 is expressed by Th1 cells that are actively recruited to sites of infection [11]. β-chemokines may block HIV-1 entry by binding to CCR5 and making it inaccessible for use by the virus [12]. The interaction of β-chemokines with CCR5 also leads to cellular activation [3] and enhanced production of HIV-1 by HIV-1–infected cells [13]. Thus, the dynamics of chemokines and their receptors may be critical to activation and/or expansion of HIV-1 infection during coinfections such as TB.

Our observations indicate that MTB induced production of MIP-1α in PBMC from all 4 study groups (HIV-1/PTB, HIV-1/C, and PTB patients and control subjects). However, MIP-1α levels were higher in both PTB groups than in the control groups. By contrast, MTB-induced MIP-1α levels were significantly lower in HIV-1/PTB and HIV-1/C patients than in the PTB and control groups. In addition, the pattern of production of RANTES, another β-chemokine, in MTB-stimulated PBMC was similar to that of MIP-1α production. Therefore, it appears that there is a limitation in MTB-induced production of β-chemokines in patients with HIV infection, regardless of the presence of PTB. Low production of β-chemokines by mononuclear cells at sites of MTB infection in HIV-1/PTB patients may be conducive to expansion of HIV-1 in situ. We recently observed a limitation in production of MIP-1α by bronchoalveolar lavage cells from patients with HIV infection, as compared with healthy subjects (H.A., unpublished data).

When we compared the effect of MTB on induction of both CCR5 and MIP-1α mRNA in PBMC of HIV-1/PTB and HIV-1/C patients, we found a predominant effect on enhancement of expression of CCR5 in dually infected patients. Expression of CCR5 mRNA was 2-fold higher in HIV-1/PTB than in HIV-1/C patients. Thus, as mononuclear cells are recruited to sites

of active MTB infection, an up-regulation of CCR5 may be conducive to increased HIV-1 infection of target cells and may eventually culminate in increased virus load. Higher expression of CCR5 on mononuclear cells of HIV-1–infected TB patients has also been reported [15].

Only in HIV-1/PTB patients with low CD4 cell counts (<500 cells/μL) did we see a significant increase in MTB-induced HIV p24 production in PBMC. Significantly higher MTB-induced MIP-1α production also was seen in this group of patients, compared with that in the HIV-1/C group. Both MIP-1α and TNF-α transcriptionally induce HIV in mononuclear cells [7, 13]. Since the CCR5 domains that mediate HIV-1 entry and cellular activation differ [16], it is possible that concentrations of β-chemokines that are too low to inhibit HIV-1 entry are adequate to allow for the activation of cells. Further studies should define the contribution of these molecules in enhanced HIV production. In conclusion, the dysregulations in β-chemokine pathways may affect the immunopathogenesis of HIV-1 differently in dually infected patients—depending on the level of immunodeficiency.

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