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Short Communication

Influence of Active Tuberculosis on Chemokine and Chemokine Receptor Expression in HIV-Infected Persons

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ABSTRACT

Tuberculosis (TB) is the major opportunistic infection of HIV-1-infected patients in developing countries. Concurrent infection with TB results in immune cells having enhanced susceptibility to HIV-1 infection, which facilitates entry and replication of the virus. Cumulative data from earlier studies indicate that TB provides a milieu of continuous cellular activation and irregularities in cytokine and chemokine circuits that favor viral replication and disease progression. To better understand the interaction of the host with HIV-1 during active tuberculosis, we investigated *in vivo* expression of the HIV-1 coreceptors, CCR5 and CXCR4, and circulating levels of the inhibitory β-chemokines, macrophage inflammatory protein-1-α (MIP-1α), macrophage inflammatory protein-1-β (MIP-1β), and regulated upon activation T cell expressed and secreted (RANTES), in HIV-positive individuals with and without active pulmonary tuberculosis. We found a significant decrease from normal in the fraction of $CD4^+$ T cells expressing CCR5 and CXCR4 in individuals infected with HIV. However, CCR5 and CXCR4 expression did not differ significantly between HIV patients with and without tuberculosis. Higher amounts of MIP-1α, MIP-1β, and RANTES were detected in plasma of HIV-1-positive individuals, particularly those with dual infection, although the increase was not found to be statistically significant.

INTRODUCTION

A NUMBER OF INFECTIOUS DISEASES often encountered during the course of human immunodeficiency virus (HIV) infection have been reported to enhance HIV-1 replication and disease progression.[1–5] Tuberculosis (TB) is one of the most important cofactors accelerating HIV disease progression toward acquired immunodeficiency syndrome (AIDS).[6] The mechanisms by which TB leads to augmentation of HIV-1 load and activity are not fully understood. A number of different mechanisms have been proposed to explain this phenomenon. These include augmentation in production of proinflammatory cytokines [tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)] in the course of TB to generate a microenvironment enhancing the productive infection of local lymphocytes by HIV,[7] the constitutive degradation of IκB-α, the major cytoplasmic inhibitor of nuclear factor κB (NF-κB), in freshly isolated peripheral blood mononuclear cells (PBMC) and monocytes from patients with TB,[8] activation of mitogen-activated protein (MAP) kinase pathways,[9] and dysregulations in β-chemokines[10] and their receptors.[11]

Chemokines are potent leukocyte activators and chemoattractants. Macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and regulated upon activation normal T cell expressed and secreted (RANTES) are the major β-chemokines produced in response to *Mycobacterium tuberculosis* infection.[12] Chemokines are the natural ligands for chemokine receptors, CCR5 and CXCR4, which can also act as HIV coreceptors. M-tropic HIV-1 isolates use CCR5 as coreceptor early in the course of HIV infection, whereas T cell tropic viruses use CXCR4 for entry into $CD4^+$ T cells, typically in a later stage of infection. *In vitro* studies have demonstrated that β-chemokines inhibit

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HIV-1 by blocking the C–C chemokine receptor-5 (CCR5), which has been identified as a necessary coreceptor for M-tropic strains of HIV-1.[13] However, the role of β-chemokines in HIV-1 disease progression *in vivo* remains unclear.

Infection with *M. tuberculosis* is endemic in India and TB is the most common opportunistic infection in HIV-1–seropositive individuals in this country. Therefore, we felt it is important to determine the effects mediated by *M. tuberculosis* infection on profiles of β-chemokines and their receptors in HIV-1-infected individuals, and explore whether their dysregulation is associated with the higher HIV-1 activity seen in HIV patients with concurrent tuberculosis.

MATERIALS AND METHODS

Study population

The study population was composed of 28 HIV-seropositive patients recruited from the HIV/TB Clinics of the Tuberculosis Research Centre and the Government Hospital for Thoracic Medicine, Chennai, India. Half of these individuals (14) also had active pulmonary TB, while the other half (14) had no evidence of lung disease based on physical and chest radiological examination. A group of 10 HIV-seronegative patients with active pulmonary TB was also included in the study. The control group consisted of 10 healthy volunteers. Each group was composed of equal numbers of men and women, with ages ranging from 23 to 57 years. The study were approved by the Institutional Research and Ethics Committees of the Tuberculosis Research Centre and written informed consent was obtained from all subjects before enrollment.

The diagnosis of tuberculosis was based on sputum smear examination for acid-fast bacilli and confirmed by culture for *M. tuberculosis*. HIV was diagnosed using two rapid tests (HIV Tridot, J. Mitra, India; CombAids, Span Diagnostics, India), and a positive result was confirmed by a third test (Western blot, J. Mitra, India). None of the patients were receiving antituberculosis or antiretroviral treatment at the time of intake into the study. Of venous blood 10 ml was collected in EDTA-coated vacutainers (Becton Dickinson) from each of the study subjects. Hemoglobin levels as well as total and differential white blood cell counts were determined for all samples using an automated hematology analyzer (ABX, France).

Phenotypic analysis of cell populations

Surface expression of CCR5 and CXCR4 on mononuclear cells in whole blood was assessed by immunostaining and flow cytometry. In brief, 100 μl of whole blood was incubated with combinations of monoclonal antibodies against CD3, CD4, CCR5, and CXCR4, at 4°C for 30 min. Mouse isotype IgG_1 and IgG_2 antibodies were used as control for nonspecific staining. All antibodies were conjugated with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE) and purchased from Becton Dickinson (Mountain View, CA). Red blood cells were lysed using 1× RBC lysis buffer. Cells were washed twice in ice-cold phosphate-buffered saline (PBS) and fixed in 500 μl of 1% paraformaldehyde for flow cytometric analysis. Samples were acquired using a FACSort flow cytometer (Becton Dickinson) and data were analyzed employing the Cell Quest software. A minimum of 10,000 lymphocytes was used for analysis.

Measurement of chemokines

Plasma levels of MIP-1α, MIP-1β, and RANTES were quantitated by ELISA using commercially available kits (Biosource immunoassay kit, Biosource International, Belgium), following the manufacturer's protocol. The detection limits of the assays were 6 pg/ml for MIP-1α, 15.6 pg/ml for MIP-1β, and 31.5 pg/ml for RANTES.

Statistical analysis

Data are presented as mean ± SEM (standard error of mean). Comparison between groups was performed using the Mann–Whitney *U* test and correlations were made by using the Spearman's test. A value of $p < 0.05$ was considered statistically significant. The Statistical Package for Social Sciences, version 8.0 (SPSS Inc, Chicago, IL) was used for all statistical analysis.

RESULTS

Expression of HIV coreceptors on PBMC

To investigate the influence of active TB on HIV coreceptor expression in HIV patients, we determined expression of CCR5 and CXCR4 in HIV patients with and without TB, and compared them with HIV-negative TB patients and healthy controls (Table 1). The percentages and absolute numbers of circulating T ($CD3^+$) cells and helper ($CD4^+$) T cells in HIV-negative TB patients did not differ from those in healthy controls. The mean $CD3^+$ and $CD4^+$ T cell count in HIV negative TB patients was 1315 ± 84 and 525 ± 37, respectively. Healthy controls had a mean $CD3^+$ and $CD4^+$ T cell count of 1385 ± 62 and 687 ± 45, respectively. However, percentages and numbers of $CD3^+$ and $CD4^+$ T cells were significantly decreased in the HIV-positive groups as compared to the HIV-negative groups, irrespective of the presence or absence of tuberculosis. The mean $CD3^+$ and $CD4^+$ T cell count in individuals with HIV alone was 702 ± 25 and 143 ± 12, respectively. Those with both HIV and TB had mean $CD3^+$ and $CD4^+$ T cell counts of 726 ± 31 and 151 ± 15, respectively.

Since mean fluorescence intensity (MFI) is believed to be a better parameter than percent positive cells for the analysis of cells that give a continuum of fluorescence intensity, we also analyzed our data using MFI and found very good correlation between MFI and percent positive cells. We have therefore represented our data as percent positive cells. Greater proportions of T cells expressed CXCR4 as opposed to CCR5. For example, 37.8% of $CD3^+$ leukocytes expressed CXCR4, whereas only 29.2% of these cells expressed CCR5. Also, CXCR4 was expressed on a greater percentage of $CD4^+$ cells (18.2%) than CCR5 (6.7%).

Expression of CCR5 was significantly reduced from normal on total T ($CD3^+$) cells of persons with HIV-1, with pulmonary TB, and those with both infections (29.2 ± 4.5 vs. 11.3 ± 2.9, 15.6 ± 1.4, and 12.1 ± 2.7, respectively) ($p < 0.05$). When the $CD4^+$ subsets of T cells were analyzed, no significant difference could be observed in CCR5 expression between TB pa-

TABLE 1. CCR5 AND CXCR4 EXPRESSION ON $CD3^+$ AND $CD4^+$ CELLS IN THE STUDY GROUPS[a]

Cell type	*HIV^+TB^+*[b] *(n = 14)*	*HIV^+TB^-*[c] *(n = 14)*	*HIV^-TB^+*[d] *(n = 10)*	*HIV^-TB^-*[e] *(n = 10)*
$CCR5^+$ $CD3^+$ cells	11.3 ± 2.9[f]	12.1 ± 2.7[f]	15.6 ± 1.4[f]	29.2 ± 4.5
$CCR5^+$ $CD4^+$ cells	1.6 ± 0.5[f]	2.5 ± 0.8[f]	8.1 ± 2.5	6.7 ± 1.4
$CXCR4^+$ $CD3^+$ cells	30.9 ± 7.4	36.5 ± 5.7	24.6 ± 4.4	37.8 ± 5.5
$CXCR4^+$ $CD4^+$ cells	5.7 ± 1.2[f]	9.6 ± 2.6[f]	13.6 ± 2.1	18.2 ± 3.0

[a]Expressed as mean percent positive cells ± SEM.
[b]HIV^+TB^+, HIV-positive patients with active tuberculosis.
[c]HIV^+TB^-, HIV-positive individuals without active tuberculosis.
[d]HIV^-TB^+, HIV-negative TB patients.
[e]HIV^-TB^-, healthy controls.
[f]$p < 0.05$ compared to HIV^-TB^-.

tients and healthy donors. On the other hand, a significant decrease was observed in the fraction of $CD4^+$ T cells expressing CCR5 in the HIV-positive groups (1.6 ± 0.5 and 2.5 ± 0.8) as compared to the HIV-negative groups (8.1 ± 2.5 and 6.7 ± 1.4) ($p < 0.05$). However, no difference was found in the proportion of $CD3^+$ and $CD4^+$ T cells expressing CCR5 between HIV patients with and without tuberculosis.

CXCR4 expression on total T ($CD3^+$) cells did not differ significantly based on disease groupings. However, analysis of the $CD4^+$ subset of T cells revealed that CXCR4 expression was significantly decreased in the HIV-positive groups (5.7 ± 1.2 and 9.6 ± 2.6) when compared to the HIV-negative groups (13.6 ± 2.1 and 18.2 ± 3.0) ($p < 0.05$). Between the two HIV-positive groups, the one composed of individuals with concurrent tuberculosis had a smaller proportion of $CD4^+$ T cells expressing CXCR4 as compared to the TB negative group. Though the decrease was about 2-fold, the difference was not found to be statistically significant.

Production of chemokines in HIV and TB

To assess whether the β-chemokine profile is specific to HIV-positive TB patients, or common to HIV in the absence of tuberculosis infection, we measured three β-chemokines, MIP-1α, MIP-1β, and RANTES, in HIV patients with and without active TB, and compared them with appropriate controls (Table 2). While individuals with either HIV or TB had plasma levels of MIP-1α similar to those of healthy controls, patients with both infections had elevated MIP-1α levels. Increased plasma levels of MIP-1β were observed in both HIV and TB patients when compared to healthy controls. Interestingly, very high levels of plasma MIP-1β were detected in HIV-negative TB patients (82.8 ± 42.4 pg/ml). A comparable amount of RANTES was detected in the plasma of patients with either infection alone. On the other hand, those dually infected with HIV and TB had elevated levels of circulating RANTES. Though the increase in circulating MIP-1α, MIP-1β, and RANTES levels in the dually infected group was not statistically significant in comparison with controls, a trend toward increased production of these chemokines was obvious in HIV patients with active TB, indicating that HIV and *Mycobacterium tuberculosis* may have an additive effect on β-chemokine production. No other correlations were found between chemokine concentrations.

Correlation of HIV coreceptor expression and chemokines production

We analyzed the relationship between chemokine production and chemokine receptor expression in $CD4^+$ cell count-matched (<200 cell/μl) groups of HIV-positive TB patients and HIV patients without TB. No significant correlation could be demonstrated between the two groups.

DISCUSSION

The importance of the copathogenesis of HIV and TB as an area of intense investigation hinges on the enormity of these in-

TABLE 2. PLASMA CHEMOKINE LEVELS IN THE STUDY GROUPS[a]

Chemokine	*HIV^+TB^+*[b] *(n = 14)*	*HIV^+TB^-*[c] *(n = 14)*	*HIV^-TB^+*[d] *(n = 10)*	*HIV^-TB^-*[e] *(n = 10)*
MIP-1α	5.6 ± 1.5	4.0 ± 1.6	2.6 ± 0.7	2.4 ± 1.0
MIP-1β	52.9 ± 16.6	43.1 ± 8.4	82.8 ± 42.4	20.6 ± 0.1
RANTES	2274.4 ± 434.1	1558.7 ± 254.1	1767.1 ± 376.1	1616.5 ± 486.2

[a]Expressed as pg/ml ± SEM.
[b]HIV^+TB^+, HIV-positive patients with active tuberculosis.
[c]HIV^+TB^-, HIV-positive individuals without active tuberculosis.
[d]HIV^-TB^+, HIV-negative TB patients.
[e]HIV^-TB^-, healthy controls.

tertwined pandemics for world health, and the bidirectional interaction between these two pathogens. HIV and *M. tuberculosis* not only cocirculate throughout the developing world, but each has contributed to prevalence and mortality caused by the other. On one hand, HIV-1 infection predisposes to the development of active TB, and, on the other hand, the course of HIV-related immunodeficiency is worsened by active *M. tuberculosis* infection.[14,15]

M. tuberculosis infection in HIV-1-infected individuals enhances both systemic[3] and local[16] viral replication, as well as HIV transmission to T cells.[17] Both relative risk of death and rates of development of new opportunistic infections are increased in HIV/TB coinfection, compared with CD4 cell-matched HIV-1-infected subjects without TB.[18] *In vivo* studies have shown that active TB increases plasma HIV levels, which decreases once anti-TB treatment is initiated.[3] Furthermore, HIV patients with active TB show evidence of immune activation, including elevated levels of proinflammatory cytokines.[19,20] These combined data suggest that *M. tuberculosis* may influence the biological variants of HIV that are expressed in HIV-infected persons with active TB.

The association of CCR5 and CXCR4 with HIV infection has been clearly demonstrated. Apart from their role as coreceptors for docking and subsequent viral entry into the host cells, CCR5 and CXCR4 are also involved in HIV-1-induced apoptosis.[21] Thus, chemokine receptor expression is a critical parameter for numerous processes. β-Chemokines, which are the natural ligands of HIV coreceptors, are thought to have a dual role in HIV-1 infection. By binding to CCR5, they may inhibit HIV-1 infection of target cells,[22] but may also induce HIV-1 replication by acting as mediators of inflammation, favoring the accumulation of activated immune cells at the foci of HIV replication, thereby exposing them to viral infection.[23] This makes knowledge of HIV coreceptor expression and β-chemokine production during concurrent infection a clinically important issue. The present study attempts to investigate whether the presence of active tuberculosis infection induces additional dysregulations in *in vivo* chemokine receptor expression and β-chemokine production in HIV patients.

A number of studies have addressed the distribution and expression of CCR5 and CXCR4 on various immune cells of HIV-1-infected persons,[24–26] and it has been suggested that the differential expression of these HIV-1 coreceptors has important implications for HIV-1 tropism and pathogenesis *in vivo*. HIV-1 isolates from individuals with early asymptomatic infection are commonly nonsyncytium inducing (NSI) and utilize CCR5 as the major coreceptor. In contrast, syncytium inducing (SI) isolates are found in individuals with more advanced disease and are associated with an accelerated loss of $CD4^+$ lymphocytes and the acquisition of opportunistic infections.[27–28] This switch in coreceptor usage has been well described for individuals infected with HIV-1 subtype B, but data from India, Ethiopia, and Malawi have indicated that CXCR4-using isolates are rarely found in individuals infected with HIV-1 subtype C, even at advanced stages of disease.[29–32] Morris *et al*.[33] reported that HIV-1 subtype C isolates from patients with active tuberculosis used CCR5 as the major coreceptor for cellular entry. Since HIV-1 subtype C is the most rapidly spreading viral subtype, accounting for more than 50% of incident infections worldwide, further analysis and monitoring of the phenotypic properties of HIV subtype C are therefore necessary and justified. We therefore felt it necessary to determine the relative levels of $CD4^+$ cells expressing CCR5 and CXCR4, and to measure circulating levels of β-chemokines in individuals living in an area where HIV-1 subtype C circulates and where TB is endemic.

We observed that although there was no significant change in CXCR4 expression on total T ($CD3^+$) cells based on disease groupings, there was a significant decline in the fraction of $CD4^+$ T cells expressing CXCR4 in HIV-infected individuals as compared to those without HIV infection. Recently, a number of studies have shown downregulation of CXCR4 in HIV-1 infected persons.[25,34,35] Forster *et al*.[25] found that CXCR4 expression on T cells in HIV-1-seropositive individuals was equally down-regulated in both $CD4^+$ and $CD8^+$ T populations. Ostrowski *et al*.[34] studied patients with other forms of chronic immune activation, but did not detect a reduction in the expression of CXCR4 on $CD4^+$ T cells, and therefore suggested that the reduction in CXCR4 expression may be relatively specific to HIV-1 infection.

We also found reduced expression of CCR5 on total T cells ($CD3^+$) in persons with TB, with HIV infection, and those with both HIV and TB as compared to healthy controls. However, analysis of the $CD4^+$ subset of T cells showed a significant decline in CCR5 expression in HIV patients alone. Our results contrast with those of Ostrowski *et al*.[34] who demonstrated that CCR5 expression was upregulated on $CD4^+$ T cells of HIV-1-infected individuals when compared to uninfected controls. Yet another group found the expression of CCR5 in HIV-1-infected patients to be significantly increased on both $CD4^+$ and $CD8^+$ T cells.[36] However, Shalekoff *et al*.[35] in their study on chemokine receptor expression in patients with HIV and TB found that CCR5-expressing $CD4^+$ T cells were not elevated in any of the disease groups. The variability in findings could be related to differences in the genetic constitution of the populations studied, the stage of disease, and the presence of other opportunistic infections in the study subjects, since surface expression of CCR5 and CXCR4 among individuals is complex and most likely regulated by multiple factors.

We observed that the presence of concomitant TB in HIV patients did not result in significant changes in CCR5 and CXCR4 expression on circulating $CD3^+$ and $CD4^+$ T cells. On the other hand, Toossi *et al*.[37] demonstrated increased expression of CCR5 on both $CD4^+$ T cells and macrophages in pleural fluid from patients coinfected with HIV-1/TB. Fraziano *et al*.[11] also found increased expression of CCR5 in human monocyte-derived macrophages and alveolar macrophages in the course of *in vivo* and *in vitro M. tuberculosis* infection. Further, Rottman *et al*.[38] reported evidence that an increased number of CCR5-positive monocytes accumulate in the lungs of patients in the course of active TB, and these sites of active *M. tuberculosis* infection in subjects coinfected with HIV-1/TB act as epifoci of HIV replication and evolution, independent of systemic HIV-1 activity. It could therefore be possible that chemokine receptor expression at sites of *M. tuberculosis* infection might be different from that observed systemically.

M. tuberculosis is known to induce synthesis and secretion of numerous cytokines and chemokines that are potentially capable of modulating HIV-1 infection and/or replication.[39,40] We found that HIV patients with active TB had higher plasma lev-

els of MIP-1α when compared to HIV-positive TB-negative patients as well as healthy controls. RANTES showed a pattern similar to MIP-1α. However, MIP-1β levels were elevated in both HIV and TB patients when compared to healthy controls. Though the increase in circulating MIP-1α, MIP-1β, and RANTES levels in the HIV-positive TB group was not statistically significant in comparison with the control group, we observed a definite trend toward increased production of these chemokines in HIV patients with active TB, indicating that HIV and *M. tuberculosis* may have an additive effect on β-chemokine production. However, since asymptomatic HIV-positive controls were not included in this investigation, the relative contribution of infection with HIV and TB to chemokine concentrations could not be obtained with certainty from our measurements in HIV-seropositive TB patients.

The elevated β-chemokine production in AIDS patients suggests that these chemokines do not have a protective role against disease progression either by their ability to competitively inhibit HIV interaction with its coreceptor on $CD4^+$ T cells, or by a protective mechanism based on their chemotactic activities which presumably regulate immune cells trafficking and inflammation. Saha *et al.*[41] demonstrated that $CD4^+$ clones from nonprogressor patients and $CD8^+$ clones from AIDS patients secreted high levels of RANTES, MIP-1α, and MIP-1β. In contrast, $CD4^+$ clones from AIDS patients produced no RANTES and little or no MIP-1α or MIP-1β. These results indicate that β-chemokine production by $CD4^+$ T cells but not $CD8^+$ T cells may constitute one mechanism of disease-free survival for HIV-1-infected individuals. Several recent studies have suggested that soluble factors other than β-chemokines, produced by $CD8^+$ T cells, can play an important role in suppressing HIV-1 replication.[42–46] It is therefore also possible that in spite of the presence of elevated levels of β-chemokines, these individuals fail to produce sufficient amounts of other HIV-1-suppressing factors, and thus fail to prevent HIV-1 replication and disease progression.

In a recent prospective study of patients with HIV/TB coinfection, TB was found to exert its most significant effect on lowering survival rates in subjects with more preserved immunological status (that is, CD4 cell counts >200 cells/μl).[47] Since the HIV-positive group in the present study was composed of individuals with CD4 cell counts <200 cells/μl, it could be possible that the effect of coinfection with *M. tuberculosis* could not be clearly appreciated in these subjects. Therefore, it appears that whereas at the site of *M. tuberculosis* infection, induction of HIV-1 expression is demonstrable at all CD4 levels, the impact of TB on HIV-1 systemically is most pronounced when patients have higher CD4 counts.

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. Further analysis of the multifactorial interaction between HIV-1 and *M. tuberculosis* may lead to novel approaches to effective drug and immune interventions.

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