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Disturbed Secretory Capacity for Macrophage Inflammatory Protein (MIP)-1α and MIP-1β in Progressive HIV Infection

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ABSTRACT

The protective role of β-chemokines in HIV infection and disease remains controversial. Contradictory findings have been reported possibly as the result of different β-chemokine detection methods. To test this, peripheral blood lymphocytes from treatment-naive HIV patients, patients on highly active antiretroviral therapy (HAART), and uninfected controls were assessed for intracellular β-chemokine levels in comparison with levels of β-chemokine secretion in culture supernatants. HIV patients had significantly higher intracellular levels of macrophages inflammatory protein (MIP)-1α and MIP-1β than uninfected control subjects. In contrast, MIP-1α and MIP-1β supernatant levels were significantly lower in HIV patients than in controls. Interestingly, both intracellular and supernatant levels of RANTES (regulated on activation, normal T cell expressed and secreted) were significantly increased in HIV patients. Prolonged (>3 years) administration of HAART in HIV patients normalized the intracellular levels of MIP-1β and RANTES and restored the decreased supernatant levels of MIP-1α and MIP-1β to levels observed among controls. Significant direct correlations observed between the intracellular and the supernatant levels of β-chemokines in controls were lost in treatment-naive (except MIP-1β) and HAART-treated patients (except RANTES after >3 years of HAART). These data indicate that lymphocytes of HIV patients display a disrupted capacity to secrete the β-chemokines MIP-1α and MIP-1β, which may constitute a mechanism of immune dysfunction in progressive HIV infection. Furthermore, we demonstrated that the detection of β-chemokines in HIV patients by different methods may indeed result in contradictory findings.

INTRODUCTION

THE β-CHEMOKINES MACROPHAGE INFLAMMATORY PROTEIN (MIP)-1α, MIP-1β, and RANTES (regulated on activation, normal T cell expressed and secreted) have been shown to be potent HIV-1 suppressor factors *in vitro*.[1] The antiviral effect of β-chemokines is mediated by blocking and down-regulation of the natural β-chemokine receptor CCR5, which is the major coreceptor of macrophage-tropic HIV-1 strains.[2] However, the role of β-chemokines in controlling HIV replication *in vivo* remains controversial. Higher levels of MIP-1α and MIP-1β in the supernatant of stimulated lymphocyte cultures were associated with asymptomatic HIV-1 infection.[3,4] In contrast, increased intracellular MIP-1β levels were rather associated with advanced HIV disease[5,6] and frequencies of β-chemokine-positive lymphocytes analyzed this way correlated positively with HIV-1 load and inversely with $CD4^+$ T cell counts.[7] The use of different β-chemokine detection methods in these studies could have led to the conflicting conclusions. Thus, the contradictory findings may suggest a disturbed relationship between the intracellular expression and the effective secretion of β-chemokines in HIV patients.

To test these hypotheses, levels of intracellular β-chemokine expression were compared to levels of effectively secreted β-chemokines by using the methods that previously generated conflicting conclusions.[4,7] Results obtained for uninfected controls were compared to those for treatment-naive HIV patients and patients on highly active antiretroviral therapy (HAART).

MATERIALS AND METHODS

Study population

Twelve treatment-naive and 13 HAART-treated HIV patients consulting at the Institute of Tropical Medicine were en-

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rolled in the study. Thirteen age-matched HIV-seronegative laboratory workers were included as controls. The study was approved by the institutional review board from the Institute of Tropical Medicine. Written informed consent was given by all study subjects prior to enrollment.

Laboratory methods

Whole blood was drawn from patients and controls into EDTA tubes. HIV-1 viral load was quantified in plasma by the Amplicor HIV-1 Monitor assay, version 1.5 (Roche). Peripheral blood mononuclear cells (PBMC) were separated from fresh whole blood by gradient centrifugation using lymphocyte separation medium (Amersham Biosciences) and resuspended at 1×10^6 cells/ml in RPMI 1640 containing L-glutamine (Biowhittaker), 100 U/ml penicillin, 100 μg/ml streptomycin (Roche), and 10% fetal calf serum (Biochrom KG).

Intracellular staining for β-chemokines

Intracellular β-chemokine detection was performed as described before.[7] Briefly, freshly isolated PBMC were cultured for 5 hr with 0.02 μg/ml phorbol myristate acetate, 1 μg/ml ionomycin, and 10 μg/ml brefeldin A (Sigma) for the detection of MIP-1α and MIP-1β, and overnight with 1.5 μg/ml monensin (Sigma) for the detection of RANTES. PBMC were surface stained using antibodies against CD3 and CD8 (Becton Dickinson), and then stained intracellularly using antibodies against RANTES, MIP-1α (Pharmingen), or MIP-1β (R&D Systems). All antibodies were fluorochrome labeled. Cells were analyzed using a FACScan flow cytometer and CellQuest software (Becton Dickinson). Intracellular β-chemokine levels were expressed as percentages of β-chemokine-positive cells within gated lymphocyte, $CD4^+$ (as $CD8^-$), and $CD8^+$ T cell subsets.

β-Chemokine supernatant secretion

β-Chemokine supernatant secretion was analyzed by using the method described by Cocchi *et al.*[4] In brief, fresh PBMC at 1×10^6 cells/ml were cultured with 1 μg/ml phytohemagglutinin (Abbott) and 10 ng/ml recombinant human interleukin-2 (IL-2) (R&D Systems) for 3 days. Supernatants were collected and stored at -80°C. β-Chemokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using antibody pairs and recombinant standards from Pharmingen for RANTES, and from R&D Systems for MIP-1α and MIP-1β.

Statistical analysis

Differences between groups were tested for statistical significance by the Mann–Whitney *U* test. Correlation analyses were performed by the Spearman's rank test. The level of significance was set at $p < 0.05$.

RESULTS

Characteristics of the study population

HIV-1 load among the 12 treatment-naive HIV patients reached a median value of 4.2 $\log_{10}$ copies/ml (range 3.4–5.9) and was below 2.6 $\log_{10}$ copies/ml in all 13 patients on HAART. To study the effect of long-term HAART on β-chemokine levels, patients on HAART were arbitrarily subdivided in one group of eight patients with < 3 years of treatment (median, 28 months; range, 3–34 months) and one group of five patients with ≥ 3 years of treatment (median, 73 months; range, 46–89 months). Median $CD4^+$ T cell count among patients on HAART < 3 years was 331/μl (range, 167–812), which was lower than among patients on HAART ≥ 3 years (median, 777/μl; range, 536–1073; $p = 0.019$) and treatment-naive patients (median, 555/μl; range, 302–1144; $p = 0.025$).

HIV patients showed discordant changes in intracellular and supernatant levels of MIP-1α and MIP-1β, but not RANTES, compared to controls

Lymphocytes from HIV patients showed significantly increased intracellular levels of MIP-1α (patients on HAART $\geq$ 3 years, $p = 0.055$) and MIP-1β (patients on HAART < 3 years, $p = 0.03$) compared to lymphocytes from controls (Fig. 1A). In contrast, HIV patients showed significantly decreased culture supernatant levels of MIP-1α (treatment-naive patients, $p = 0.03$; patients on HAART < 3 years, $p = 0.007$) and MIP-1β (treatment-naive patients, $p = 0.011$) compared to controls (Fig. 1B). For RANTES, both intracellular levels (treatment-naive patients, $p = 0.039$; patients on HAART < 3 year, $p = 0.002$; Fig. 1A) and supernatant levels (patients on HAART $<$ 3 years, $p = 0.006$; patients on HAART ≥ 3 years, $p = 0.002$; Fig. 1B) were significantly increased among HIV patients. The significantly increased intracellular MIP-1β and RANTES levels and the significantly decreased MIP-1α and MIP-1β supernatant levels among HIV patients were normalized to control levels after prolonged ($\geq$3 years) HAART. Comparable results were obtained for intracellular β-chemokine levels studied within $CD4^+$ T cells and $CD8^+$ T cells (data not shown).

Intracellular β-chemokine levels correlated with levels of β-chemokine secretion in uninfected controls but not in HIV patients

Uninfected controls showed direct correlations between intracellular β-chemokine levels in lymphocytes and β-chemokine supernatant levels for MIP-α, MIP-1β, and RANTES (Fig. 2). Among treatment-naive patients, such correlation was observed for MIP-1β only (Fig. 2). No correlations between intracellular and supernatant β-chemokine levels were found among HAART-treated patients (Fig. 2), except for RANTES within the group of HIV patients on HAART ≥ 3 years ($p < 0.001$). Comparable results were obtained for intracellular β-chemokine levels studied within $CD4^+$ T cells and $CD8^+$ T cells (data not shown).

Correlation of intracellular and supernatant β-chemokine levels with markers of HIV disease progression

Among HAART-treated patients, $CD4^+$ T cell counts inversely correlated with intracellular lymphocyte levels of MIP-1β ($r = -0.637$, $p = 0.019$) and RANTES ($r = -0.687$, $p = 0.01$). No statistically significant correlations were observed be-

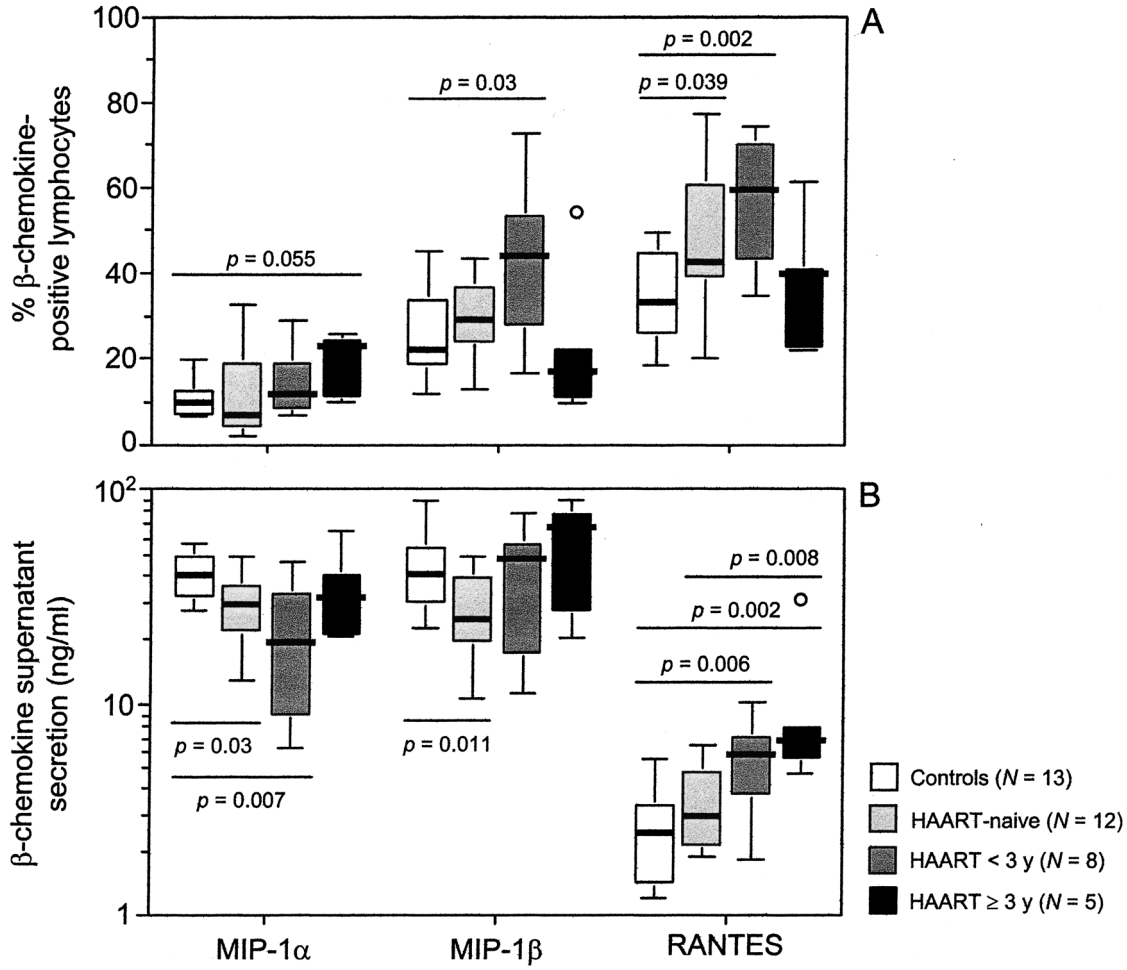

FIG. 1. Intracellular β-chemokine levels and levels of β-chemokine secretion among controls, treatment-naive, and HAART-treated HIV patients. Percentages of β-chemokine-positive lymphocytes detected by intracellular staining (**A**) and levels of β-chemokine secretion in PBMC culture supernatants detected by ELISA (**B**) were analyzed for MIP-1α, MIP-1β, and RANTES. Box plots represent the lowest value, 25th, 50th (median), and 75th percentiles, and the highest value indicated by horizontal lines in ascending order. Differences between groups were tested for statistical significance by the Mann–Whitney *U* test. *p*, level of significance.

tween levels of HIV-1 viral load and intracellular or supernatant levels of β-chemokines in any of the subject groups.

DISCUSSION

Contradictory findings concerning the protective role of β-chemokines in HIV infection and disease have been reported possibly as a result of the use of different detection methods. To address this issue, we compared intracellular β-chemokine levels in different lymphocyte subsets with levels of secreted β-chemokines in lymphocyte culture supernatants from HIV patients and controls. We found that increased intracellular MIP-1α and MIP-1β levels in lymphocytes from HIV patients were reflected by decreased levels of MIP-1α and MIP-1β supernatant secretion. A disrupted secretory capacity for MIP-1α and MIP-1β in HIV patients may explain previous contradictory reports, and may contribute to the progressive immune dysfunction during HIV infection.

Our data are in agreement with our previous study analyzing intracellular β-chemokine levels in Abidjan, Côte d'Ivoire,[7] although differences in the levels of intracellular MIP-1α and MIP-1β between HIV patients and controls were more pronounced in the latter study. Our results also confirm findings from others showing decreased levels of MIP-1α and MIP-1β supernatant secretion in symptomatic HIV infection,[4] notwithstanding that all HIV patients in that study were HAART treated and β-chemokine levels in treatment-naive patients were not studied. Thus, in the light of our current findings, the reported low MIP-1α and MIP-1β supernatant levels[4] and high intracellular MIP-1α and MIP-1β levels[7] in progressive HIV infection may have represented only apparently contradictory data. Both studies do support our findings of a disturbed capacity to effectively secrete β-chemokines in progressing HIV patients. Together, these studies suggest that high levels of intracellular

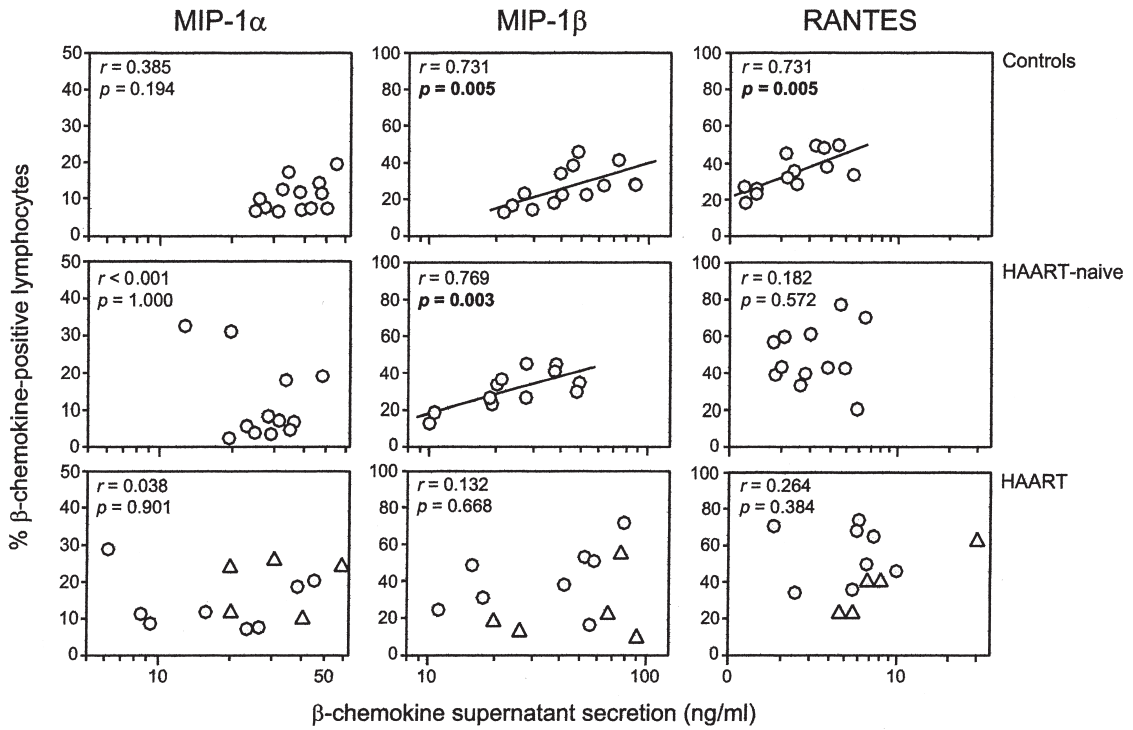

FIG. 2. Correlations between intracellular β-chemokine levels and levels of β-chemokine secretion among controls, treatment-naive, and HAART-treated HIV patients. Percentages of β-chemokine-positive lymphocytes detected by intracellular staining (y-axes) were correlated to levels of β-chemokine secretion in PBMC culture supernatants detected by ELISA (x-axes) for RANTES, MIP-1α, and MIP-1β. Data are shown for 13 HIV-uninfected controls, 12 treatment-naive HIV patients, and 13 patient on HAART (○ for HAART $<$ 3 years; △ for HAART $\geq$ 3 years). Correlation analyses were performed by Spearman's rank test. r, correlation coefficient; p, level of significance. Significant p values are displayed in bold.

MIP-1α and MIP-1β accumulation together with low levels of MIP-1α and MIP-1β secretion may constitute a correlate of HIV disease progression.

Our results confirm and extend previous data in HIV patients showing an inverse association between intracellular and serum levels of MIP-1β,[6] reduced interferon (IFN)-γ bulk secretion together with an overexpanded IFN-γ-positive cell subset,[8] and decreased supernatant secretion of IL-15 despite normal mRNA levels.[9] Furthermore, our data are also in agreement with HIV-induced impairment of inhibitory substances other than cytokines or β-chemokines. Patients with progressive HIV infection showed decreased perforin and granzyme production by natural killer cells and $CD8^+$ T cells,[10–12] and had decreased levels of the CD8 antiviral factor.[13]

A disrupted secretory capacity for MIP-1α and MIP-1β may provide yet another mechanism of HIV pathogenesis. Increased intracellular β-chemokine levels in $CD4^+$ and $CD8^+$ T cells have been associated with viremia-driven chronic T cell activation.[7] Together with an increased T cell activation state, a decreased capacity to effectively secrete HIV-suppressive MIP-1α and MIP-1β in the vicinity will render these $CD4^+$ T cells even more susceptible to productive HIV infection and replication.

In contrast with our observation for MIP-1α and MIP-1β, both intracellular and secreted RANTES levels were found to be increased in HIV patients, suggesting that the secretory capacity of RANTES remained intact. In agreement with this discordance, RANTES was recently found to be rapidly secreted from intracellular granules containing presynthesized proteins, while MIP-1α and MIP-1β secretion depended on a retarded release of newly synthesized proteins.[14] Therefore, our data may indicate that only the latter secretory pathway, and its dependence on immediate Golgi processing, is disrupted during HIV infection.

Interestingly, intracellular levels of MIP-1β and RANTES and supernatant levels of MIP-1α and MIP-1β were normalized in HIV patients with $\geq$ 3 years of HAART. This coincided with higher $CD4^+$ T cell counts in the latter group compared to those on HAART for $<$ 3 years and treatment-naive patients, and with inverse correlations between $CD4^+$ T cell counts and intracellular levels of MIP-1β and RANTES among HAART-treated patients. These findings are in line with previous studies showing normalization of MIP-1α and MIP-1β supernatant levels in HIV patients by antiretroviral treatment.[15,16] In addition, our observations confirm that dysfunctional immune responses in HIV patients may be restored after prolonged periods of antiretroviral treatment only, as was recently proposed for other immunological parameters than β-chemokines.[17]

In summary, we have shown that lymphocytes of HIV patients display a disturbed capacity to secrete the β-chemokines MIP-1α and MIP-1β, which may constitute a mechanism of immune dysfunction in progressive HIV infection. The data sug-

gest that the putative role of β-chemokines in protection against HIV disease progression may depend on the preservation of an intact β-chemokine secretory profile.

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