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Human and Simian Immunodeficiency Virus-Infected Chimpanzees Do Not Have Increased Intracellular Levels of β-Chemokines in Contrast to Infected Humans

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This study was undertaken to explain why chimpanzees infected with HIV-1 (human immunodeficiency virus type 1) or SIV_{cpz} (simian immunodeficiency virus of chimpanzee) are relatively resistant to AIDS (acquired immunodeficiency syndrome). The numbers of β-chemokine-positive cells were compared between uninfected and infected humans and chimpanzees using three-color cytofluorometry. In humans, the percentage of β-chemokine-positive cells was significantly higher in $CD8^+$ T and natural killer (NK) cells than in $CD4^+$ T cells in both uninfected and HIV-1-infected individuals. In the presence of HIV-1 infection, however, both $CD8^+$ and $CD4^+$ T cell subsets contained significantly more β-chemokine-positive cells than in the absence of infection. Interestingly, in chimpanzees two important differences were noted. First, their percentage of β-chemokine-positive $CD8^+$ T and NK cells was significantly higher than in uninfected humans. Second, in contrast to humans, infection with either HIV-1 or with SIV_{cpz} was not associated with increased numbers of β-chemokine-positive cells. These results indicate that: constitutive high levels of intracellular β-chemokines in chimpanzees' CD8 lymphocytes and NK cells do not necessarily correspond to lower levels of virus replication during the chronic phase of infection; and increased percentages of β-chemokine-positive cells in HIV-infection are not a correlate of disease resistance. The data suggest that neither pre-nor post-exposure levels of intracellular β-chemokines are correlated with the subsequent control of disease progression. ***J. Med. Virol. 69:297–305, 2003.*** © 2003 Wiley-Liss, Inc.

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INTRODUCTION

Chimpanzees infected with human immunodeficiency virus type 1 (HIV-1) and the closely related simian immunodeficiency virus of chimpanzee (SIV_{cpz}) [Gao et al., 1999], develop only rarely the acquired immunodeficiency syndrome (AIDS) [Zarling et al., 1990; Heeney, 1995; Novembre et al., 1997; Villinger et al., 1997; Koopman et al., 1999]. Because of their close genetic relationship with humans, infected chimpanzees are uniquely relevant to study the immune mechanisms accounting for resistance to disease progression.

The reasons for the different susceptibility of humans and chimpanzees to AIDS are not clear but several hypotheses have been put forward [Heeney et al., 1993, 1997; Balla Jhagjhoorsingh et al., 1999; Norley et al., 1999]. Virus replication may be suppressed more efficiently in chimpanzees than in humans possibly by the induction of soluble non-cytolytic suppressive factors. These substances might confer some anti-viral activity on the $CD8^+$ T or NK cells toward infected CD4

The study protocols were approved by the Ethical Review Boards of the Institute of Tropical Medicine and the BPRC and were implemented according to international guidelines. All patients provided informed consent for their participation in this study.

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lymphocytes [Fehniger et al., 1998; Oliva et al., 1998] or some degree of resistance to virus replication on the $CD4^+$ lymphocytes [Furci et al., 1997; Rosenberg et al., 1997; Maeda et al., 1999]. In HIV-1-infected humans, various potent soluble non-cytolytic inhibitors of HIV-1 replication in vitro have been identified over the years, mainly produced by the $CD8^+$ T cell [Walker et al., 1986; Brinchmann et al., 1990]. These suppressor factors include the $CD8^+$ cell antiviral factor (CAF) and β-chemokines such as "regulated on activation normal T expressed and secreted" (RANTES), macrophage inflammatory protein-1α (MIP-1α) and MIP-1β [Cocchi et al., 1995; Levy et al., 1996]. These β-chemokines are predominantly active against macrophage-tropic non syncytium-inducing (NSI) HIV-1 strains and suppress HIV-1 by blocking fusion and virus entry via the β-chemokine receptor CCR5 on monocytes and $CD4^+$ T cells [Cocchi et al., 1995; Jansson et al., 1996; Oravecz et al., 1996; Leith et al., 1997; Mackewicz et al., 1997]. $CD8^+$ T cells from asymptomatic HIV infected individuals have been described to secrete higher levels of β-chemokines [Clerici et al., 1996; Ullum et al., 1998; Garzino-Demo et al., 1999] and to suppress virus replication more vigorously than $CD8^+$ T cells from patients with clinical AIDS [Walker et al., 1989; Mackewicz et al., 1991; Blackbourn et al., 1996]. Vigorous β-chemokine production has also been identified as a true correlate of protective immunity in human and macaque vaccine trials [Moss et al., 1997, 1998; Wang et al., 1998]. Yet the actual role of β-chemokine as correlate of resistance to disease remains controversial. Although some researchers observed a correlation between increased level of β-chemokine secretion, protection against infection and slower progression to the disease [Schwartz et al., 1997; Ullum et al., 1998; Zagury et al., 1998; Wasik et al., 1999], other studies did not report evidence for such an association [McKenzie et al., 1996; Zanussi et al., 1996; Kreuzer et al., 2000]. Investigations in SIV_{cpz}-infected chimpanzees and in vaccinated macaques were equally contradictory.

Despite the clear inhibition capacity of recombinant β-chemokines on SIV_{cpz} and SIVmac replication in vitro, endogenous β-chemokines did not appear to be the only factors implicated in the $CD8^+$ T cell-mediated suppression of viral replication in vitro. [Kestens et al., 1995; Gaudin et al., 1998; Ondoa et al., 2002].

The apparent lack of clear correlation between β-chemokines and protection against AIDS may be explained by the small effect that global β-chemokine secretion in the plasma has on disease progression. Moreover, the measure of plasma or serum levels of β-chemokines as a marker for lymphocyte production can be highly inaccurate due to spontaneous release by activated cells despite careful sample collection [Klinger et al., 1995; Bubel et al., 1996]. Also, in vitro assays evaluating β-chemokine release in cell culture supernatants may be too artificial and may not always correspond with the actual intracellular concentration of β-chemokine [Tartakovsky et al., 1999]. A more inxformative approach would be to investigate the relationship between protective immunity and the differential intracellular expression of β-chemokines in specific subsets of peripheral blood mononuclear cells (PBMCs).

The present study was undertaken to address further the issue of β-chemokine production and susceptibility to HIV disease. We hypothesized that high intracellular level of β-chemokines correlate with protective immunity in HIV-1/SIV_{cpz} infection of chimpanzees and humans. A cohort of HIV-1 and SIV_{cpz} infected chimpanzees, which are naturally refractory to AIDS, was compared to groups of HIV-1 infected humans, with respect to their capacity to produce β-chemokines. Groups of non-infected chimpanzees and humans acted as controls. The impact of HIV-1 and SIV_{cpz} RNA viral loads in the plasma of infected human and chimpanzees on the number of circulating cells expressing intracellular β-chemokines was also examined.

MATERIAL AND METHODS

Patients

Fresh ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood samples were obtained from 19 HIV-1 consecutive seropositive patients and from 21 virus negative healthy controls. The HIV-1 seropositive subjects had a mean $CD4^+$ T cell percentage of 18 (range, 6–36%) and a mean $CD4^+$ T cell count of 396 (range, 138–643) cells per μl blood. Thirteen patients were under treatment with a combination of protease inhibitors and nucleoside reverse transcriptase inhibitors at the time of study whereas six patients had never received treatment. The six untreated HIV-1 seropositive subjects had a mean $CD4^+$ T cell percentage of 21 (range, 7–36%) and an absolute $CD4^+$ T cell count of 422 (range, 166–679) cells per μl. The 13 treated HIV-1 seropositive subjects had a mean $CD4^+$ T cell percentage of 15 (range, 6–31%) and an absolute $CD4^+$ T cell count of 407 (range, 138–643) cells per μl. All HIV seropositive subjects are outpatients at the Institute of Tropical Medicine in Antwerp. All HIV seronegative control blood samples were provided by the Antwerp blood transfusion center.

Chimpanzees

The chimpanzees included in this study are part of an HIV-1-infected cohort housed at the Biomedical Primate Research Center (BPRC) [Balla Jhagjhoorsingh et al., 1999]. Three chimpanzees (Ch-Bu, Ch-Ma and Ch-Co) were infected experimentally with HIV-1 between 1982 and 1984. One chimpanzee (Ch-No) was infected naturally with $SIV_{cpz\text{-}ant}$ [Vanden Haesevelde et al., 1996] and was diagnosed since 1989 [Peeters et al., 1992] and another (Ch-Ni) was infected experimentally with peripheral blood mononuclear cells (PBMCs) from Ch-No in October 1995. Four healthy uninfected animals served as controls. Fresh EDTA peripheral blood was obtained from these nine chimpanzees during the study period in 1998.

Detection of Intracellular β-Chemokine Expression in Lymphocytes From Patients and Chimpanzees

For the intracellular detection of RANTES, freshly obtained EDTA whole blood was washed twice with HBSS (Gibco BRL, Life Technologies, Scotland) and a third time with RPMI 1640 (Gibco BRL) containing 10% bovine calf serum (BCS from HyClone, Logan, UT) and reconstituted to its original volume. Briefly, 100 μl of washed blood was diluted 1/5 with RPMI 1640 and 10% BCS. Subsequently, 2.5 μl monensin (2 μM/ml, final concentration, Sigma, Brussels) was added and the cells were left overnight at 37°C and 5% CO_2 in the incubator. Intracellular detection of MIP-1α and MIB-1β was carried out on PBMCs, purified from fresh EDTA whole blood by density gradient centrifugation and which were kept overnight in RPMI 1640 containing 10% BCS at a concentration of 1×10^6 PBMCs/ml at 37°C in 5% CO_2 before use. Subsequently, 20 μl Phorbol myristyl acetate (PMA, 20 ng/ml final concentration), 20 μl ionomycin (1 μg/ml final concentration) and 10 μl brefeldin-A (10 μg/ml final concentration), all purchased from Sigma (Brussels, Belgium) were added to 1 ml of PBMC and left to incubate at 37°C and 5% CO_2 for 5 hr. Cell surface staining was either on whole blood (RANTES) or on PBMCs (MIP-1α and MIB-1β) by washing the cells with phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA from Acros, NJ) and by incubating the cells with 5 μl of monoclonal anti-CD3-PerCP and CD8-FITC or CD8-PE (Becton-Dickinson, Belgium) for 15 min at room temperature (RT). The whole blood specimen was treated with 2ml of lysing solution from Becton-Dickinson (Erembodegem, Belgium) for 10 min in the dark to remove the red blood cells, centrifuged and washed with PBS-1% BSA. PBMCs and lysed whole blood were fixed with 100 μl of Cytoperm™ (Serotec, UK) reagent A for 15 min at RT, washed and permeabilized with 100 μl of reagent B for 30 min at RT. Subsequently, permeabilized cell aliquots were washed and incubated for 15 min at RT with either PE-conjugated monoclonal antibodies to RANTES and MIP1α (both from Pharmingen, Becton Dickinson), or with carboxyfluorescein-conjugated monoclonal antibody to MIP-1β (R&D Systems, Abington, UK). Finally, cells were washed in PBS-1% BSA and fixed with 1% paraformaldehyde in PBS and analyzed by FACScan (Becton Dickinson).

Plasma RNA Viral Load Determination in Infected Patients and Chimpanzees

The HIV-1 RNA viral load in HIV-1 infected humans and chimpanzees was determined using the commercially available COBAS Amplicor HIV-1 Monitor test version 1.5 (Roche, Indianapolis, IN). This ultrasensitive method was used according to the manufacturer instructions, with a linear detection range between 50 and 50,000 copies/ml. The SIV_{cpz} plasma RNA viral load test has been described previously [ten Haaft et al., 1995, 1998, 2001] and was based on a competitive RT-PCR in the pol region, which is conserved between $SIV_{cpz\text{-}ant}$ and HIV-1.

Statistical Analysis

Differences between two groups were tested for statistical significance using the non-parametric Mann-Whitney *U*-test. Bivariate correlations were measured using the non-parametric Spearman rank correlation test. Alpha was set at 0.05.

RESULTS

β-Chemokine-Positive T Lymphocytes and NK Cells in Uninfected Humans and Chimpanzees

Intracellular expression of β-chemokines was measured in $CD4^+$ and $CD8^+$ T cells, and in an NK cell subset ($CD8^+$ $CD3^-$ lymphocytes). Representative examples of the flow cytometric analysis of intracellular β-chemokine expression in an infected and an uninfected subject is presented in Figure 1 (human) and Figure 2 (chimpanzee). RANTES is expressed constitutively and can be measured without the need for prior mitogenic stimulation. Therefore, intracellular expression of RANTES was measured ex vivo in unstimulated whole blood samples as a measure of spontaneously produced RANTES. In uninfected humans, the median percentage of each lymphocyte subset, expressing RANTES was 10% for $CD4^+$ T cells, 47% for $CD8^+$ T cells and 71% for $CD8^+$ $CD3^-$ NK cells (Fig. 3). In contrast to RANTES, MIP-1β and MIP-1α expression was low or undetectable in unstimulated cells and they were only measured after in vitro stimulation of PBMCs with PMA and Ionomycin as a measure "stimulation-induced" β-chemokine positive cells.

In uninfected humans, the median percentage of MIP-1α- and MIP-1β-positive cells was, like RANTES, higher in the $CD8^+$ T cell subset (15 and 24% respectively) than in the $CD4^+$ T cell subset (3 and 3% respectively). The highest percentage of positive cells however, were found in the $CD8^+$ $CD3^-$ NK cell subset (50 and 79% respectively). Taken together, human NK cells were more likely to β-chemokine-positive than $CD4^+$ and $CD8^+$ T cells. To study possible associations between the expression levels of the three β-chemokines, we calculated the non-parametric Spearman rank correlation coefficients in uninfected human controls. Significant correlations were found between the percentage of MIP-1α- and MIP-1β-positive $CD4^+$ T cells ($r = 0.79$; $P < 0.001$) and between MIP-1α- and MIP-1β-positive $CD8^+$ T cells ($r = 0.62$; $P = 0.002$). Similarly, significant correlations were found between the percentage of RANTES- and MIP-1β-positive $CD8^+$ T cells ($r = 0.73$; $P < 0.001$) and between RANTES- and MIP-1β-positive $CD4^+$ T cells ($r = 0.69$; $P = 0.001$).

In uninfected control chimpanzees, the percentage of RANTES-, MIP-1α- and MIP-1β-positive cells was, as in uninfected humans, significantly higher in $CD8^+$ T

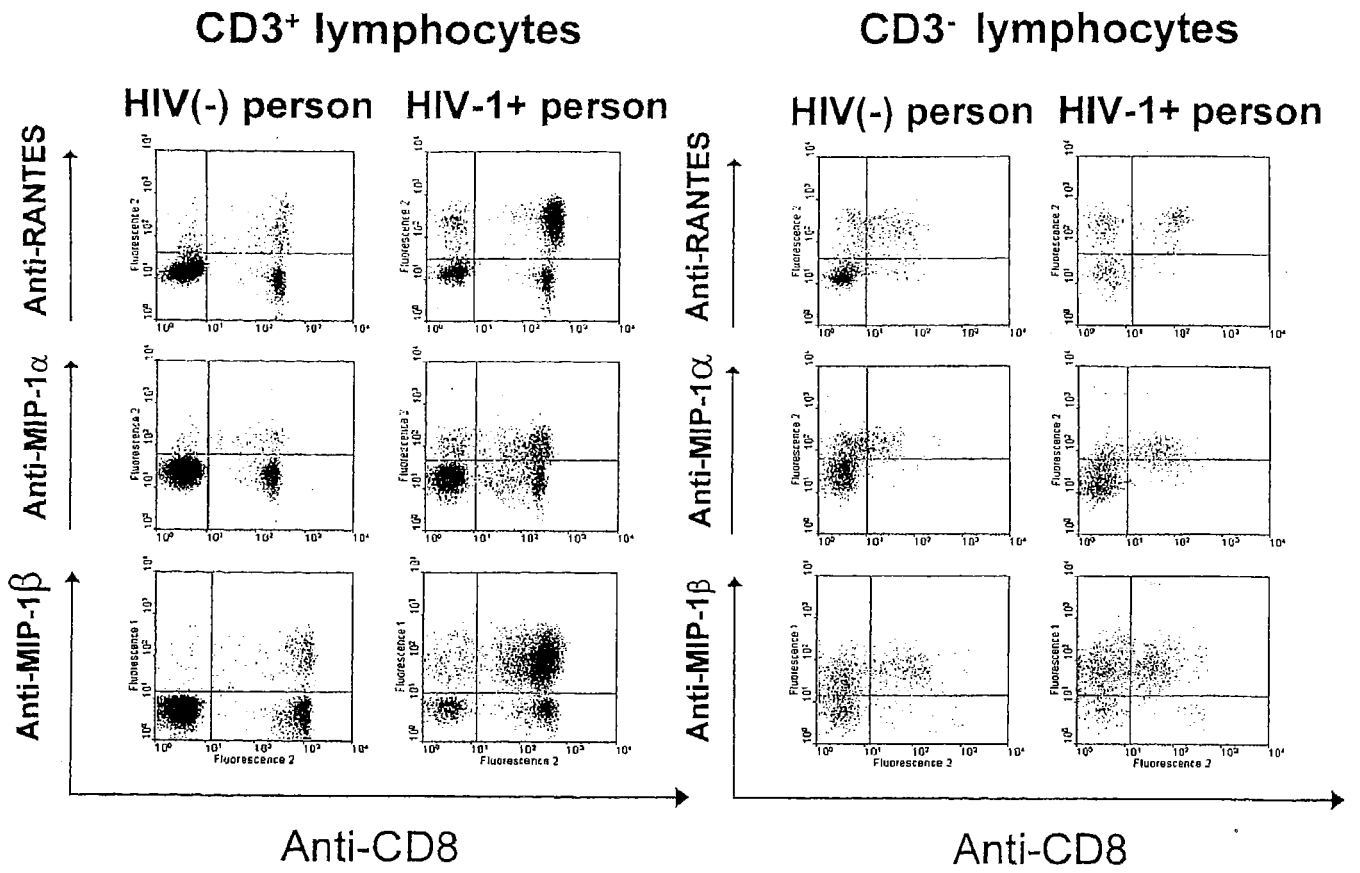

Fig. 1. Two representative examples of intracellular detection of RANTES, MIP-1α and MIP-1β in T cells ($CD3^+$ lymphocytes) and $CD8^+$ NK cells (CD3-negative lymphocytes) from an HIV-1 infected person and an uninfected controls subject. RANTES was detected ex vivo in unstimulated whole blood and represents constitutive RANTES production. MIP-1α and MIP-1β were measured after in vitro stimulation with phorbol myristyl acetate and ionomycin where $CD3^+CD8^-$ lymphocytes were considered as $CD4^+$ T cells, $CD3^+CD8^+$ lymphocytes as $CD8^+$ T cells and $CD3^-$ $CD8^+$ lymphocytes as $CD8^+$ NK cells. Of note is that $CD8^+$ T lymphocytes and $CD8^+$ NK cells are potent producers of β-chemokines and that HIV-1 infection is associated with elevated percentages of β-chemokine-positive cells within the $CD4^+$ and $CD8^+$ T cell subsets.

cells (80, 36 and 65% respectively) and $CD8^+$ $CD3^-$ NK cells (91, 75 and 81% respectively) than in $CD4^+$ T cells (<10%) (Fig. 3). These results have been described in part in a separate study [Ondoa et al., 2002]. The $CD8^+$ T cell subset and $CD8^+$ $CD3^-$ NK cell subset from uninfected chimpanzees contained more RANTES-positive cells than the corresponding humans subsets ($P = 0.009$ and $P = 0.0045$ respectively) (Fig. 3). Similarly, the percentage of MIP-1α-positive cells was higher in the chimpanzee $CD8^+$ T cell subset and in the $CD8^+$ $CD3^-$ NK cell subset but also in $CD4^+$ T cell subset than in their human counterparts ($P = 0.014$, $P = 0.016$ and $P = 0.042$ respectively). Finally, the percentage of MIP-1β-positive cells was higher in chimpanzee $CD8^+$ T cells ($P = 0.016$) and chimpanzee $CD4^+$ T cells ($P = 0.042$) but not in $CD8^+$ $CD3^-$ NK cells ($P = 0.63$) than in uninfected human cells.

HIV-1 Infection is Associated With Increased Expression of β-Chemokines in Humans But Not in HIV-1 or $SIV_{cpz\text{-}ant}$-Infected Chimpanzees

The percentage of β-chemokine-positive lymphocytes was significantly higher in HIV-1-infected humans than in uninfected controls (Fig. 3). The relative increase of β-chemokine positive cells was most pronounced in the $CD4^+$ T cell subset where the percentage of RANTES ($P < 0.001$) and MIP-1β ($P < 0.001$) expressing cells tripled and that of MIP1α doubled ($P = 0.014$). Within the $CD4^+$ T cell subset, the percentage of RANTES-positive cells correlated very well with the percentage of MIP-1β-positive cells ($r = 0.86$, $P < 0.001$, data not shown) and to a lesser extent with the percentage of MIP-1α-positive cells ($r = 0.60$, $P = 0.015$). In addition, the percentage of RANTES- and MIP-1β-positive cells within the $CD4^+$ T cell subset correlated significantly but inversely with the total percentage of $CD4^+$ T cells (RANTES: $r = -0.78$, $P < 0.001$; MIP-1β: $r = -0.65$, $P = 0.003$). In other words, in HIV patients with low $CD4^+$ T cell counts, the relative number of $CD4^+$ T cells expressing RANTES and MIP-1β was significantly higher than in patients with higher $CD4^+$ T cell counts and less advanced disease. Within the $CD8^+$ T cell subset from HIV-1-infected humans, the percentage of β-chemokine-positive cells increased from 47–86% for RANTES ($P < 0.001$), from 24–51% for MIP-1β ($P < 0.001$) and from 15 to 29% for MIP-1α ($P = 0.019$) (Fig. 3). The percentage of β-chemokine expressing cells

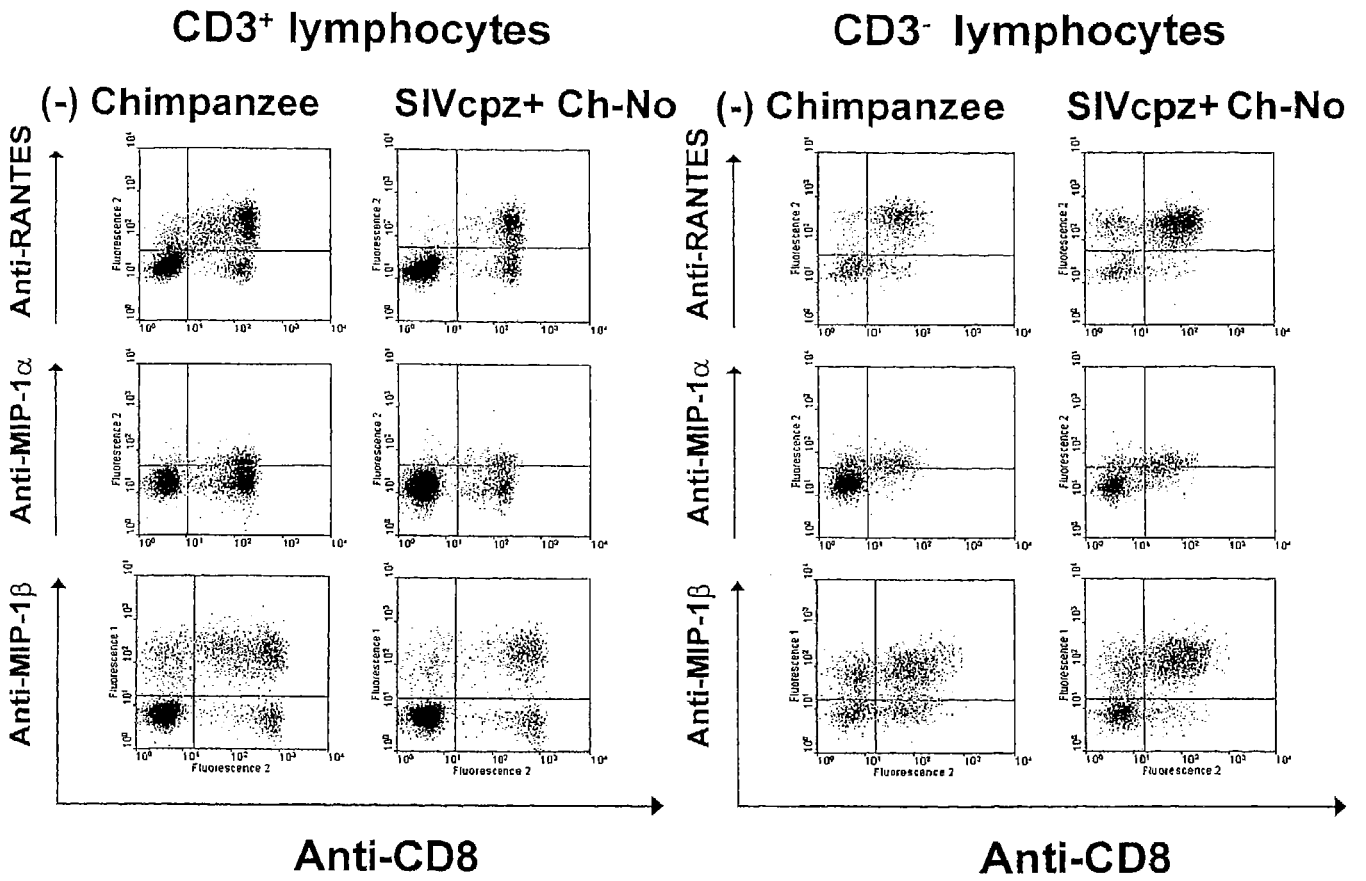

Fig. 2. Two representative examples of intracellular detection of RANTES, MIP-1α and MIP-1β in CD8$^+$ T cells (CD3$^+$ CD8$^+$ lymphocytes), in CD4$^+$ T cells (CD3$^+$ CD8-lymphocytes), and in CD8$^+$ NK cells (CD3$^-$CD8$^+$ lymphocytes) of a SIV$_{cpz\text{-}ant}$-infected chimpanzee (Ch-No) and an uninfected control chimpanzee. The detection of β-chemokines was done as described in Figure 1 and in Materials and Methods. Of note is that CD8$^+$ T lymphocytes and CD8$^+$ NK cells are potent producers of β-chemokines and that SIV$_{cpz}$ (and HIV-1) infection is not associated with elevated percentages of β-chemokine-positive CD4$^+$ and CD8$^+$ T lymphocytes.

within the CD8$^+$ NK cell subset however, was not significantly different from that of uninfected controls. In contrast to the observations in uninfected controls, no significant correlations were observed between the percentages of RANTES, MIP-1α and MIP-1β chemokine expressing CD8$^+$ T cells in HIV seropositive subjects. To investigate whether the percentage of β-chemokine-positive cells in HIV-1-infected humans was a consequence of HIV-1 infection itself, rather than of HAART treatment, we compared the percentage β-chemokine-positive cells between untreated and HAART treated patients. The results indicated that the percentage β-chemokine-positive cells per subset were at least as high, if not higher in untreated HIV-1 patients than in the treated patients. The differences between the HAART-treated and untreated group however, were not statistically significant (Fig. 4). This shows that the augmented percentage of β-chemokine-positive cells in HIV-1-infected humans is primarily a consequence of infection and not of treatment.

In contrast to HIV-1-infected humans, intracellular β-chemokine expression in CD4$^+$ T cells, CD8$^+$ T cells and CD8$^+$ NK cells did not differ between HIV-1- or SIV$_{cpz\text{-}ant}$-infected chimpanzees and uninfected control chimpanzees (Fig. 3). These results have been partially described in a separate study [Ondoa et al., 2002].

RNA Viral Load Determinations in HIV-1 Infected Patients and HIV-1/SIV$_{cpz}$-Infected Chimpanzees

All six untreated HIV-1 infected patients had detectable HIV-1 plasma RNA viral loads [median log RNA/ml: 4.22 (range 3.26–4.84), data not shown]. Of the 13 HAART-treated patients, four had a detectable viral load (median log RNA/ml: 3.32 [range 2.75–3.77)]. All HIV-1 infected chimpanzees had undetectable HIV-1 RNA viral loads (<1.7 log copies/ml). The two SIV$_{cpz}$-infected animals had positive viral loads; one Ch-No had a viral load of 5.04 log copies/ml whereas the other had a viral load of 4.27 log copies/ml. To explore the association between the percentage of chemokine positive cells and on the one hand and HIV-1 log RNA viral load on the other, we carried out a non-parametric Spearman rank correlation analysis. No significant correlations could be detected, however (correlation coefficient was <0.2 for each analysis).

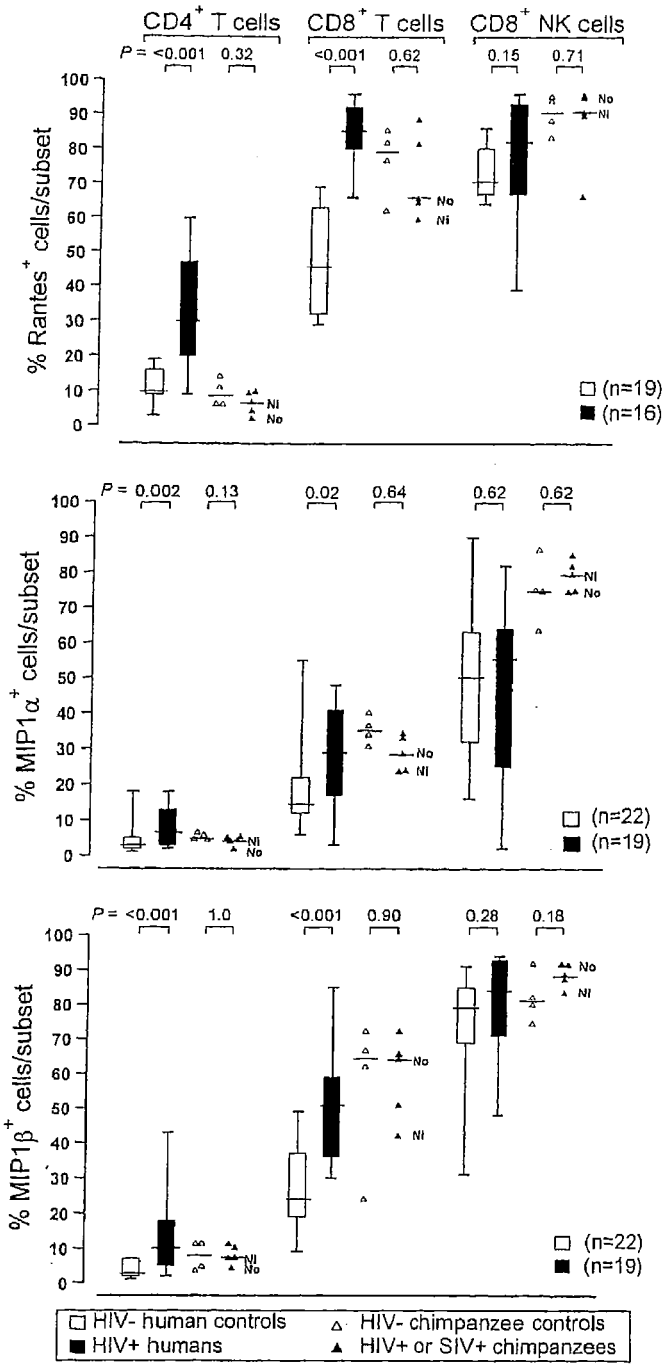

Fig. 3. Comparison of the intracellular detection of RANTES (top graph) in whole blood and MIP-1α (middle graph) and MIP-1β (bottom graph) in PBMC of 19 HIV-1 seropositive patients and 22 uninfected (HIV-) human controls, of two $SIV_{cpz\text{-}ant}$-infected chimpanzees (▲-No; ▲-Ni); 3 HIV-1-infected chimpanzees (▲); and 4 uninfected control chimpanzees (△). Results are presented as percentages of positive cells in each subset and represent median, interquartile range (boxes) and the 5–95% data range (error bars) of the human data, and as individual data points and the median of the chimpanzee data. Differences between two groups were tested for statistical significance by the non-parametric Mann-Whitney *U*-test.

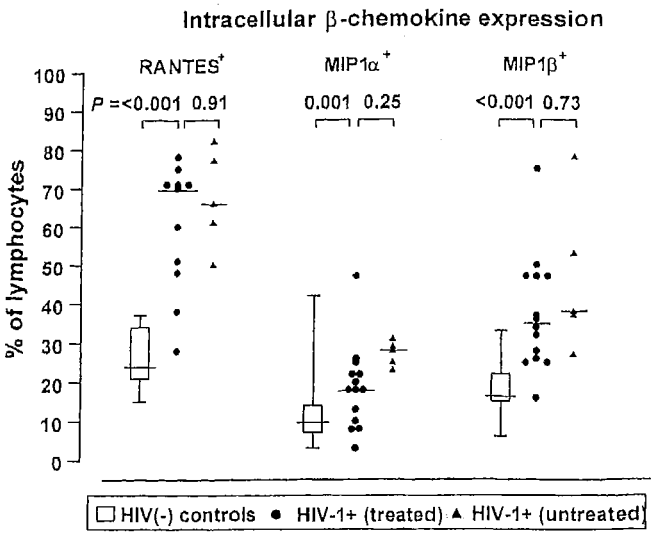

Fig. 4. The elevated percentages of MIP-1α-, MIP-1β- and RANTES positive lymphocytes (T + B + NK cells) of 22 uninfected human controls (box plot), 14 HIV-1-infected humans treated with highly active anti-retroviral therapy (●, HAART) and 5 untreated HIV-1-infected humans (▲). Intracellular expression of these β-chemokines in HAART treated HIV-1-infected subjects did not differ significantly from that of untreated HIV-1 seropositive subjects. Results are presented as percentages of positive cells in each subset and represent median, interquartile range (boxes) and the 5–95% data range (error bars) of the human HIV-1 seronegative controls, and as individual data points and the median of the untreated and treated subjects. Differences between two groups were tested for statistical significance by the non-parametric Mann-Whitney *U*-test.

DISCUSSION

In an attempt to explain the relative resistance of chimpanzees to AIDS, the number of β-chemokine-positive cells in uninfected and infected humans and chimpanzees was compared. As in humans, β-chemokines-positive lymphocytes were predominantly $CD8^+$ and much less $CD4^+$ in chimpanzees. Because substantially higher percentages of β-chemokine-positive $CD4^+$ T cells were not detected in chimpanzees as compared to humans, the hypothesis that the relative high resistance of chimpanzees to AIDS may be related to a higher endogenous production of β-chemokines by chimpanzee $CD4^+$ T cells is unlikely.

The present study showed that humans and chimpanzee $CD8^+$ T and $CD8^+$ NK cells are potent producers of β-chemokines like RANTES, MIP-1α and MIP-1β. These results are in line with previous studies that measured β-chemokine production in supernatants of resting and stimulated PBMCs [Conlon et al., 1995] and stress the importance of determining the cell phenotype associated with β-chemokine secretion. Interestingly, more $CD8^+$ T and NK cells from uninfected chimpanzees were positive for RANTES, MIP-1α and MIP-1β than their human counterparts. The fact that NK cells are the most potent producers of β-chemokines combined with the observation that chimpanzees have constitutively three to 10 times more NK cells than humans [Ondoa et al., 2002], make this cell subset an important source of β-chemokines in vivo. Although it is difficult to assess

the physiological significance of these difference between humans and chimpanzees it is tempting to speculate that chimpanzee $CD8^+$ T and NK cells may have a better potential to suppress the replication of β-chemokine susceptible virus strains their human counterparts cells. Our previous investigations did not produce any consistent evidence that endogenous soluble β-chemokines from infected chimpanzees were a major factor of the $CD8^+$ T cell–mediated in vitro suppression of SIV_{cpz} replication [Ondoa et al., 2001, 2002]. Equally, NK cells were not found to exert a suppressive activity on SIV_{cpz} replication in vitro [Kestens et al., 1995; Ondoa et al., 2002], despite their capacity to secrete high levels of RANTES and in contradiction with observations in HIV-1 infected humans [Fehniger et al., 1998; Oliva et al., 1998]. It is not excluded that constitutive high numbers of circulating β-chemokine expressing cells, reflects important concentration of these anti-viral factors in some remote sites of virus replication within the host. High pre-infection levels of β-chemokines may play a crucial role during the initial and post-acute phase of infection. They may contribute to low viral replication set points in some critical virus reservoirs like the lymph nodes [Koopman et al., 1999]. Whether this correlates with the low pathogenicity of HIV-1 and SIV_{cpz} infection in chimpanzees remains to be clarified.

In HIV-1 infected individuals, the elevation of the percentage of β-chemokine-positive lymphocytes was at least as high in untreated as in HAART-treated patients. This indicated that the augmented relative numbers of β-chemokine-positive cells within the $CD4^+$ and $CD8^+$ T cells subsets were the direct consequence of HIV-infection rather than of HAART treatment. Furthermore, the relative increase of β-chemokine-positive cells within the $CD4^+$ and $CD8^+$ T cell subsets correlated significantly but inversely with the $CD4^+$ T cell percentage. These observations suggest further that the augmented percentage of β-chemokine-positive cells associated with HIV-1 infection in humans, is a correlate of progression rather than resistance to disease progression.

Because plasma RNA viral load is one of the best marker used to follow the evolution of the disease, the absence of a clear association between this parameter and the relative increase of β-chemokine-positive cells within the $CD4^+$ and $CD8^+$ T cells subsets in infected was unexpected. In a different study of larger cohort of untreated HIV-1-infected African subjects have indeed shown significant correlations between viral load and levels of intracellular β-chemokine expression [Jennes et al., 2002]. The fact that a successful HAART results in a dramatic decrease of plasma viral load [Katzenstein et al., 1996; Richman, 1996] as well as with a possible increase of β-chemokine production [Aukrust et al., 1998], may explain the lack of correlation between viral load and β-chemokine production by T cells in the group of HIV-infected patients.

In HIV-1-infected chimpanzees, the absence of augmented β-chemokines production was expected as the three animals consistently showed undetectable plasma viral load since their experimental infection more than 10 years ago [Ondoa et al., 2001]. This explanation is not valid for the two SIV_{cpz} infected chimpanzees from whom relatively high viral load could be regularly detected over a 6- and a 12-year-period of follow-up, respectively [Ondoa et al., 2001]. Previous investigations strongly suggest that disease progression consistently correlate with chronic cellular immune activation [Kestens et al., 1992, 1994]. It is most likely that the increase of β-chemokine production is associated with the augmented in vivo T cell activation status rather than the viral load in HIV-1-infected patients. Indeed, both $CD4^+$ and $CD8^+$ T cells subsets display increased levels of these surface activation antigens as HIV disease progresses and even with patients under HAART [Kestens et al., 1992, 1994]. Increased T cell activation is associated with increased serum RANTES mRNA levels in HIV-1-infected humans [Muller et al., 1997]. $SIV_{cpz\text{-}ant}$ infection in chimpanzees, on the other hand, is not associated with chronic systemic immune activation [Gougeon et al., 1997; Kestens et al., 1998] despite significant plasma viral load and does not correspond to increased levels of β-chemokine production.

In conclusion, the results indicate that on the one hand chimpanzees $CD8^+$ T and NK cells express constitutively more β-chemokine that their human counterparts. On the other hand, HIV-1 and SIV_{cpz} infection in chimpanzees do not result in a significant increase of the percentage of cells positive for β-chemokines in contrast to HIV-1 infection in humans. These findings suggest that elevated levels of intracellular β-chemokines in $CD8^+$ T and NK cells before infection do not necessarily contribute to lower levels of virus replication in infected chimpanzees. Moreover, the data do not support the hypothesis that increased proportion of β-chemokine-positive T cells induced by HIV-infection in humans is a correlate of resistance to HIV-induced disease. Taken together, these observations do not corroborate the idea that β-chemokines significantly contribute to slow disease progression in infected human and chimpanzees.

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