RANTES Production from CD4⁺ Lymphocytes Correlates with Host Genotype and Rates of Human Immunodeficiency Virus Type 1 Disease Progression

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Several chemokine and chemokine receptor parameters were measured in peripheral blood mononuclear cells obtained from patients before they became infected with human immunodeficiency virus type 1 (HIV-1). After HIV-1 infection, the parameters were compared with plasma HIV-1 RNA levels and with rates of CD4⁺ lymphocyte decline. Patients who were heterozygous for the Δ32CCR5 allele had significantly higher levels of RANTES production from their CD4⁺ lymphocytes than did patients who did not carry the Δ32CCR5 allele (P = .01). Higher RANTES production levels from ex vivo–activated CD4⁺-enriched lymphocytes, but not CD8⁺ lymphocytes, correlated with lower plasma HIV-1 RNA levels 9–12 months after infection (P = .01) and with slower rates of CD4⁺ lymphocyte decline (P = .002). CCR5 expression levels on ex vivo–activated CD4⁺ lymphocytes did not correlate with markers of disease progression. These results further support the hypothesis that chemokine production levels are associated with HIV-1 replication in vivo.

A multitude of CC and CXC chemokine receptors function as viral coreceptors in conjunction with the CD4 molecule to enable human immunodeficiency virus type 1 (HIV-1) entry into target cells [1]. CCR5, the CC chemokine receptor, and CXCR4, the CXC chemokine receptor, are the 2 most significant receptors with respect to HIV-1 transmission and pathogenesis [2]. Viruses that use CCR5 (R5 viruses) are preferentially transmitted and found during the early stages of infection, whereas viruses that use CXCR4 (X4 viruses) are more likely associated with later-stage disease [2–4].

The CC chemokines RANTES, macrophage inflammatory protein (MIP)–1α, and MIP-1β are the natural ligands for CCR5, and the CXC chemokine stromal cell–derived factor–1 serves as the ligand for CXCR4 [1]. These molecules efficiently block virus entry and suppress replication of the appropriate HIV-1 strains in vitro. The R5 virus infection profile in vitro can be correlated with both CCR5 expression at the cell surface and secretion levels of CC chemokines [5, 6]. Several clinical studies highlight the ability of chemokine and chemokine receptor expression levels to affect virus replication, with low receptor expression and higher chemokine production levels favoring reduced virus replication or even protection from infection [7, 8]. Some studies, however, indicate that high CC chemokine levels enhance virus infectivity in vitro or are associated with more rapid progression to disease [9, 10]. The most compelling evidence linking chemokine and chemokine receptor parameters with disease progression is the association of polymorphisms within the genes coding for these molecules with either protection from infection or altered rates of disease progression [4]. Most striking is the 32-bp deletion within the coding region of the CCR5 coreceptor gene, Δ32CCR5, which is associated with a slower rate of progression to disease [2].

In this study, we sought to determine whether the characteristics of chemokine production or chemokine receptor expression before HIV-1 infection predicted the postinfection disease course and how these were related to the presence or absence of a Δ32CCR5 allele.

Patients and Methods

Study patient selection. On the basis of the availability of pre- and postinfection peripheral blood samples (time of infection was
We isolated CD4+ progression for each corresponding donor. For the in vitro assay findings to the postinfection markers of disease patients before the extrapolated time of infection and compared our in vitro assay findings to the postinfection markers of disease progression for each corresponding donor. For the Δ32CCR5 comparison, we also incorporated a group of uninfected control subjects. Because of variations in the number of recovered activated lymphocytes, not all parameters could be studied for all of the study patients. All data points available have been included in presentations and data analysis.

HIV-1 infectivity of CD4+ lymphocytes in vitro. We sought to determine whether there were differences in either R5 or X4 virus replication in CD4+ lymphocytes isolated before infection. There was no association between replication of R5 or X4 viruses and plasma HIV-1 RNA levels or the slope of CD4+ lymphocyte decline (data not shown). We also found no association between infectivity profiles and the different CCR5 genotypic subgroups.

Chemokine and chemokine receptor expression levels. RANTES production levels were measured from purified CD4+ and CD8+ lymphocytes. Enriched CD4+ and CD8+ lymphocytes were cultured separately at $1.0 \times 10^6$ cells/mL for 4 days after separation, and RANTES levels were determined by use of a commercially available immunoassay (R&D Systems). The CCR5 and CXCR4 cell surface expression levels were determined by fluorescence-activated cell sorter (FACS) analysis, using monoclonal antibodies 2G12 and 2D12, respectively, as described elsewhere [5]. CCR5 and CXCR4 mRNA expression levels were determined by use of real-time kinetic reverse transcriptase–polymerase chain reaction (PCR) assays. In brief, internal sequence-specific probes linked to 2 fluorescent dyes (a 5′ reporter and a 3′ quencher) were added to the PCR mix before initiating amplification of the specific target sequences. The efficiency of amplification was determined by coamplification of an exogenously added unique RNA standard of known copy number prepared by in vitro transcription with DNase treatment to ensure the absence of contaminating DNA.

Statistical analysis. Fisher’s exact (2 × 2 tables) and χ² (N × N tables) tests were used to determine the statistical significance of differences in the distribution of categorical variables between groups. The nonparametric Wilcoxon rank sum test was used to determine the statistical significance of differences in continuous variables among the different groups. Correlation among variables was evaluated by use of Spearman’s nonparametric test. Significance was established at $P < .03$.
Figure 1. RANTES production levels from CD4+ -enriched lymphocytes isolated from noninfected CC chemokine receptor 5 (CCR5) wild-type (CCR5-wt/wt) and Δ32CCR5 heterozygous patients.

Figure 2. Plasma human immunodeficiency virus–1 RNA levels (copies/mL) 9–12 months after infection (A) and decline of CD4+ cells (cells/mL/year; B) dependent on the median RANTES production level (764 pg/mL) from CD4+ -enriched lymphocytes.

Discussion

We showed previously that CC chemokine levels and CCR5 expression levels correlated with HIV-1 R5 virus infectivity in vitro and, therefore, wanted to determine whether these factors correlated with in vivo markers of disease progression [5, 6]. To do so, we analyzed several chemokine and chemokine receptor profiles in ex vivo–activated CD4+ and CD8+ cell populations generated from PBMC obtained 9–12 months before HIV-1 infection and compared the results with in vivo markers of disease progression after infection. From the parameters that we measured, we identified that patients with the higher CD4+ lymphocyte culture levels of RANTES before infection had lower plasma HIV-1 RNA levels and slower rates of CD4+ lymphocyte decline after infection. Higher levels of RANTES in the supernatants of cultured CD4+ lymphocytes also were observed in patients who were heterozygous for the Δ32CCR5 allele, a finding in support of our previous report [5].

We used pre- rather than postinfection samples, to eliminate the possibility that HIV-1 replication could skew the RANTES production profiles by altering the lymphocyte subsets being propagated in culture. Although it is unlikely, we cannot rule out the possibility that the exposure of these patients to HIV-1 antigen encountered during the period before the presumed time of infection in some way influenced our results. However, we observed no difference in the spectrum of RANTES secretion patterns between the patients who became infected and nonexposed control subjects (data not shown). In addition, mitogen activation of the cells with PHA is likely to have obscured any influence of HIV-1–specific lymphocyte responses.

No correlations were observed between virus infectivity profiles, cell surface CCR5/CXCR4 or CCR5/CXCR4 mRNA expression levels, and plasma HIV-1 RNA levels or CD4+ T lymphocyte decline. This may be a reflection on the duration and method of the stimulation employed in these experiments.

The results presented here support a number of studies describing an association between CC chemokine production levels from activated PBMC and clinical course [7, 8]. A previous study, strongly supported by our own findings, demonstrated that CD4+ lymphocyte clones, but not CD8+ lymphocyte...
clones, from long-term nonprogressing patients secreted higher levels of CC chemokines than did clones derived from rapid progressors [8]. Taken together, these studies support the concept that chemokine production from CD4\(^+\) lymphocytes can protect the lymphocytes from infection and presumably from destruction. As an added benefit, the protection of CD4\(^+\) cells by CC chemokines may allow for the maintenance of a vigorous anti–HIV-1–specific CD4\(^+\) T lymphocyte response. This may lead to better control of viral replication through the induction and maintenance of effective neutralizing antibody and cytotoxic T lymphocyte responses [13].

A recent study has reported high levels of RANTES, MIP-1\(\alpha\), and MIP-1\(\beta\) being secreted from HIV-1 antigen-specific CD8\(^+\) lymphocytes, which inversely correlates with the corresponding infected individuals’ HIV-1 load measurements [14]. Of interest, as with our own findings described here, this study showed higher cellular production levels of the CC chemokines in patients who were heterozygous for the \(\Delta32\)CCR5 allele. These results suggest that a higher production of CC chemokines may be beneficial in maintaining lower levels of virus replication through the induction of a more effective anti–HIV-1–mediated immune response. Whether patients with the higher preinfection levels of CD4\(^+\) cell CC chemokine production correspond to patients who subsequently develop higher postinfection HIV-1 antigen–specific CD8\(^+\) production levels remains to be determined. Our study adds considerable weight to the phenomenon that chemokine production levels are genetically predetermined and can correlate with the control of HIV-1 replication in vivo, although via which mechanism is still unknown.

References


