Infection (reviewed in [1]), an event associated with a rapid progression to AIDS, there is strong evidence supporting the former possibility [4]. In vitro, CCR5-binding chemokines inhibit infection by R5 strains, whereas the physiologic ligand of CXCR4, the chemokine stromal cell–derived factor (SDF-1), now designated “CXCL12” [2], inhibits infection by X4 strains (reviewed in [1, 5]). CCR5-binding chemokines are typical examples of CC-inducible chemokines, which are mainly produced in response to inflammatory stimuli, whereas SDF-1 is the paradigm of homeostatic or lymphoid chemokines (reviewed in [2, 6]), constitutively produced by bone-marrow stromal cells, lymphoid organs, and a great variety of other cells and tissues, except blood leukocytes [7–13]. Although CCR5 is physiologically dispensable, and its absence in CCR5Δ32 homozygosity provides the host with significant protection against infection by R5 strains (reviewed in [14–16]), SDF-1 and its receptor, CXCR4, which is expressed not only in hematopoietic cells but in a great variety of other cells and tissues, are physiologically indispensable [6, 9, 17–27]. The SDF-1–CXCR4 interaction is essential for the maturation of CD34+ stem cells in the bone-marrow environment, for B cell lymphopoiesis and myelopoiesis, for the physiologic traffic of lymphocytes and their endothelial transmigration, and for car-
Table 1. Demographic, virologic, and immunologic characteristics of human immunodeficiency virus–infected groups when plasma stromal cell–derived factor (SDF)–1 levels and expression of CXCR4 on T lymphocytes were evaluated.

<table>
<thead>
<tr>
<th>Group</th>
<th>Follow-up, mean ± SD, months</th>
<th>Age, mean ± SD, years</th>
<th>Sex, % male</th>
<th>Risk factor, %</th>
<th>Plasma virus load, mean ± SD copies/mL</th>
<th>CD4+ T cells/mm³, mean ± SD</th>
<th>CD8+ T cells/mm³, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTNP (n = 67)</td>
<td>135 ± 12</td>
<td>31 ± 8</td>
<td>69</td>
<td>Sexual</td>
<td>21 ± 70</td>
<td>10,830</td>
<td>696 ± 219</td>
</tr>
<tr>
<td>LVL (n = 15)</td>
<td>31 ± 20</td>
<td>36 ± 10</td>
<td>56</td>
<td>Intravenous</td>
<td>75 ± 25</td>
<td>299</td>
<td>706 ± 215</td>
</tr>
<tr>
<td>ES (n = 26)</td>
<td>40 ± 25</td>
<td>36 ± 13</td>
<td>72</td>
<td>Hemophilic</td>
<td>65 ± 35</td>
<td>99,238</td>
<td>584 ± 171</td>
</tr>
<tr>
<td>IS (n = 39)</td>
<td>160 ± 10</td>
<td>21 ± 10</td>
<td>60</td>
<td>Subjects</td>
<td>18 ± 82</td>
<td>58,138</td>
<td>345 ± 180</td>
</tr>
<tr>
<td>AS (n = 15)</td>
<td>81 ± 53</td>
<td>42 ± 8</td>
<td>100</td>
<td></td>
<td></td>
<td>884,731</td>
<td>61 ± 56</td>
</tr>
</tbody>
</table>

NOTE. LTNP, long-term nonprogressors; LVL, subjects with low plasma virus load; ES, early stage of HIV-1 infection; IS, intermediate stage of HIV-1 infection; AS, advanced stage of HIV-1 infection.

diovascular and cerebellar embryoorganogenesis, as demonstrated by mice deficient in either CXCR4 or SDF-1 genes, which die perinatally [6, 9, 17–27]. SDF-1 also has T cell costimulatory properties [28] and directs the migration of plasma cells to bone marrow [29].

Apart from CCR5Δ32 deletion, other genetic polymorphisms in chemokine and chemokine receptor genes influencing susceptibility or resistance to HIV infection and its progression to AIDS are known (reviewed in [14–16]). One of these is the SDF1-3A polymorphism, consisting of a G→A mutation at position 801 (counting from the ATG start codon) in the 3′ untranslated-region transcript of the SDF-1 gene [30]. Its homozygosity, but not its heterozygosity, has been reported to be associated with a delayed progression to AIDS and AIDS-related death [30], and, because the 3′ untranslated region is involved in regulating the stability of mRNA, it has been suggested that such homozygosity might involve an overproduction of SDF-1 that could act by inhibiting the appearance of X4 strains [30]. Other studies, however, have not confirmed the progression-retarding effects of this genotype, and some of them have found that it is, in fact, associated with both a faster decline in CD4+ T cells and progression to AIDS [31–37].

On the other hand, SDF1-3A heterozygosity was found increased in exposed but uninfected (EU) homosexual men, a finding suggesting that this genotype could play a protective role against HIV infection [30]. Thus far, no other studies have analyzed in detail the SDF1-3A polymorphism in EU adults, few have investigated plasma SDF-1 levels in relation to HIV disease [38–40], and no data exist on whether there is a relationship between plasma SDF-1 levels and SDF1-3A genotype. Whether there is a relationship between plasma SDF-1 levels and CXCR4 expression on T lymphocytes is also not known; this issue deserves attention because the SDF1-1-mediated down-regulation of surface CXCR4 expression on T lympho-

Table 2. Distribution of stromal cell–derived factor-1 (SDF-1)–3A polymorphism among human immunodeficiency virus (HIV)–infected subjects, heavily exposed but uninfected subjects, and controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypic frequency</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTNP (n = 82)</td>
<td>wt/wt 31 (37.8)</td>
<td>111 (67.68)</td>
</tr>
<tr>
<td></td>
<td>wt/3A 49 (59.8)</td>
<td>53 (32.32)</td>
</tr>
<tr>
<td></td>
<td>wt/3A 2 (2.4)</td>
<td></td>
</tr>
<tr>
<td>ES (n = 26)</td>
<td>15 (57.7)</td>
<td>41 (78.85)</td>
</tr>
<tr>
<td>IS + AS (n = 44)</td>
<td>22 (50.0)</td>
<td>62 (70.45)</td>
</tr>
<tr>
<td>EU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (n = 30)</td>
<td>25 (83.3)</td>
<td>55 (91.67)</td>
</tr>
<tr>
<td>Hem (n = 30)</td>
<td>17 (56.7)</td>
<td>43 (71.67)</td>
</tr>
<tr>
<td>Healthy controls (n = 88)</td>
<td>49 (55.7)</td>
<td>131 (74.43)</td>
</tr>
<tr>
<td></td>
<td>33 (37.5)</td>
<td>45 (25.57)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. %; wt, wild-type SDF-1 allele; 3A, mutated SDF-1-3A allele; EU, exposed but uninfected subjects (EU-sex, sexual exposure to HIV-1; EU-hem, hemophilia highly exposed to non-virus-inactivated clotting factor concentrates between 1980 and 1985); LTNP, long-term nonprogressors; ES, early stage of HIV-1 infection; IS, intermediate stage of HIV-1 infection; AS, advanced stage of HIV-1 infection (IS and AS can be considered typical progressors and, for this comparison, were considered together). Only P values <.05 are shown.

* P <.005 vs. EU-hem.
* P <.0001 vs. EU-sex.
* P <.004 vs. healthy controls.
* P <.03 vs. healthy controls.
* P <.001 vs. HIV-positive individuals.
* P <.05 vs. EU-hem.
* P <.008 vs. healthy controls.
cytes plays a major role in the in vitro antiviral effects of SDF-1 against X4 strains [41, 42]. The current study was undertaken to gain insight into these aspects.

Subjects, Materials, and Methods

Study subjects. Subjects were recruited at the Hospital Clinic (Barcelona), at the Hospital “La Fe” (Valencia), and at the “Centro Médico Sandoval” (Madrid), a specialized center for sexually transmitted diseases. These centers are public, nonprofit, and academic institutions of the National Health Service of Spain.

Three groups were studied, all of them lacking CCR5Δ32 homozygosity. The first group, HIV-1–positive subjects, was subdivided into the following categories: (1) LTNP (n = 67), defined as asymptomatic subjects with CD4+ T cell counts >500 × 10^6/L after >10 years of known seroprevalence in the absence of antiretroviral therapy (15 asymptomatic subjects with low virus load [plasma virus load <500 copies of HIV RNA/mL in 3 consecutive determinations at 5-month intervals], without antiretroviral therapy after >1 year of follow-up, included in the LTNP group [n = 82]; because the plasma virus load set point in seroprevalent subjects is the main predictor of the disease-progression rate and therefore these subjects with low virus loads can be considered as presumably future LTNP [43]); (2) early-stage asymptomatic (ES) patients (n = 26), with CD4+ T cell counts >500 × 10^6/L in the absence of antiretroviral therapy, after a follow-up period of <7 years after seroprevalence diagnosis; (3) intermediate-stage (IS) patients (n = 39), with CD4+ T cell counts 150–300 × 10^6/L after follow-up of <7 years after seroprevalence diagnosis (none of whom had developed opportunistic infections); and (4) advanced-stage (AS) patients (n = 15), with CD4+ T cell counts <100 × 10^6/L and who had developed an opportunistic infection after 5–10 years of known seroprevalence.

The second group, EU subjects (n = 60), who were seronegative and had undetectable plasma virus loads after ≥4 consecutive determinations at 6-month intervals, were subdivided into the following categories: (1) 30 subjects (EU-sex), including 23 women, 5 heterosexual men, and 2 homosexual men, who during ≥12 months prior to the present study and during the present study, had high exposure to HIV-1, through unprotected sex with HIV-infected partners, with a median of >600 episodes of intravaginal or anal intercourse with ejaculation during the 1–4-year follow-up period; these EU-sex and their HIV-positive partners mostly belong to a large cohort of subjects with high-risk sexual behavior, with a long follow-up (“Centro de Salud Sandoval,” Madrid) [44]; (2) 30 hemophiliacs (EU-hem), who were highly exposed to non–virus-inactivated clotting-factor concentrates before 1986; these EU-hem belong to a large cohort of hemophiliacs (Hospital “La Fe,” Valencia), who mostly became HIV infected [45, 46], and all of them, including the 30 EU-hem studied here, also became infected with hepatitis C virus.

The third group, healthy controls (HC) (n = 119) who were screened as blood donors, were subjects age- and sex-matched with the HIV-positive and EU groups, who had normal biologic parameters, including being negative for HIV-1, hepatitis C virus, and other infections.

Not all subjects in each group could be assessed on all parameters (the precise number for each parameter is indicated). IS and AS patients can be considered as typical progressors, and, for comparisons with regard to SDF1-3′A polymorphism, they were analyzed as a single group. Table 1 shows the demographic, virologic, and immunologic status of HIV-1–infected patients at the time when both the expression of CXCR4 and plasma SDF-1 levels were analyzed.

SDF1-3′A and CCR5Δ32 polymorphism. Genomic DNA was isolated from either peripheral-blood mononuclear cells or whole blood (Qiagen). SDF1-3′A was analyzed by polymerase chain reaction (PCR)–restriction fragment-length polymorphism analysis with primers SDF-F (5′-CAAGCTGAAGCAGTGGAC-3′) and SDF-R (5′-AGGTTTGGTCCTGAGAGTCC-3′) and the MspI enzyme (Promega), whose restriction site is eliminated by the G→A mutation of the SDF1-3′A polymorphism [30]. Heterozygotes and mutated homozygotes were tested ≥2 different times to ensure soundness of results. PCR analysis of CCR5Δ32 was performed with primers spanning the 32-bp deletion, as reported elsewhere [47].

ELISA quantification of plasma SDF-1. Plasma obtained from an EDTA-anticoagulated blood sample, obtained 16–24 h after extraction of venous blood, was kept at −80°C until required for ELISA determinations. SDF-1 ELISA was developed with appropriate pairs of coating and catcher anti–SDF-1 antibodies, as well as with standard recombinant human SDF-1α (R&D Systems). The ELISA conditions for antibody coating, blocking, reagent for
plasma dilution, and enzyme-developing reaction were as recommended by the manufacturer (R&D Systems), with ELISA high-binding capacity plates (MaxiSorb; Nunc Products). All determinations were done in duplicate, and in most cases they were confirmed in an independent experiment.

**CXCR4 expression on CD4** and **CD8** T lymphocytes.** From the same sample that had been used to obtain plasma, peripheral-blood mononuclear cells were isolated by standard Ficoll (1.077 density) gradient centrifugation and were cryopreserved 4 h after being collected [48]. The expression of surface CXCR4 on CD4** and **CD8** T lymphocytes was analyzed by flow cytometry using thawed cells and phycoerythrin–conjugated CXCR4 (Becton Dickinson), on a FACSCalibur flow cytometer (Becton Dickinson), as reported elsewhere [48]. With respect to CXCR4 expression, there were no significant differences between fresh and cryopreserved cells.

**Statistical analysis.** Proportions of different genotypes and allelic frequencies between groups were analyzed by χ² test. Plasma SDF-1 levels and percentages of CXCR4** CD4** and **CD8** lymphocytes were compared by the Kruskal-Wallis test, with Bonferroni adjustment of P being used for multiple comparisons.

**Results**

**Distribution of SDF1-3′A polymorphism.** The genotypic and allelic frequencies of SDF1-3′A polymorphism are shown in table 2. Among HC (n = 88), the SDF1-3′A allele frequency was 25.57%, with 6.8% (6/88) and 37.5% (33/88) frequencies, respectively, of SDF1-3′A homozygous and heterozygous genotypes, frequencies that are consistent with data reported in other healthy white populations [30–32, 49, 50]. With respect to SDF1-3′A allelic frequency, there were no significant differences between HIV-positive groups and HC, and this also was true for the frequency of SDF1-3′A homozygosity, although the lowest SDF1-3′A homozygosity frequency occurred in LTNP (2/82 [2.4%]) and in ES patients (0/26), whereas the highest value occurred in patients with a more advanced stage of HIV-1 infection—that is, the IS + AS group (4/44 [9.1%]; P < .01, compared with HC). There was a trend toward an increased heterozygote frequency in all HIV-1–positive groups, compared with HC, although this increase was statistically significant only for LTNP (49/82 [59.8%]; P = .004), whereas in ES patients (11/26 [42.3%] and in the IS + AS group (18/44 [40.9%]) it did not significantly differ from that in HC (table 2).

Among EU subjects, the allelic frequency (8.33%) of SDF1-3′A in EU-sex was clearly lower than that in HC (P = .008) and LTNP (P = .001); it also was lower than that in EU-hem (28.33%), although at the limit of statistical significance (P = .05), probably because of the low number of subjects. In accordance with this decreased allelic frequency, EU-sex lacked mutated homozygotes (0/30), and the SDF1-3′A heterozygote frequency (5/30 [16.7%]) was significantly lower than both that in HC (P = .03) and that in LTNP (P = .001) (table 2). In contrast, in EU-hem the SDF1-3′A allele frequency (28.33%), as well as the frequencies of the SDF1-3′A homozygous (4/30 [13.3%]) and heterozygous (9/30 [30.0%]) genotypes, did not differ significantly from those in either HC or the HIV-positive group, except for a significantly decreased heterozygote frequency compared with that in LTNP (P = .005). Although it lacked statistical significance, the SDF1-3′A homozygote fre-
Plasma SDF-1 levels. The median and interquartile range (IQR) of plasma SDF-1 concentrations in the different HIV-1–positive groups and in the EU and HC groups are shown in figure 1. The plasma SDF-1 levels in HC (n = 119) were 0.0–198 ng/mL (median, 11.2 ng/mL; IQR, 7.85–20.27). In the HIV-positive group, the medians were 29.4 ng/mL (range, 5.4–238.6; IQR, 17.4–40.8) in LTNP (n = 70), 33.3 ng/mL (range, 12.0–215.1; IQR, 16.83–46.15) in ES patients (n = 16), 33.3 ng/mL (range, 1.0–171.0; IQR, 13.3–69.6) in IS patients (n = 30), and 17.8 ng/mL (range, 2.3–65.7; IQR, 4.1–24.4) in AS patients (n = 13). Compared with those of HC, plasma SDF-1 concentrations in all these HIV-positive groups were significantly increased (P < .0005), except in AS patients (P > .72) (figure 1).

Among EU subjects, plasma SDF-1 levels in EU-sex (n = 30) were 1.2–123.7 ng/mL (median, 30.9 ng/mL; IQR, 20.3–48.6). These values were highly increased compared with those in HC (P < .0005) and EU-hem (P < .0005). The latter group, EU-hem (n = 30), showed a range of 1.3–64.1 ng/mL (median, 9.6 ng/mL; IQR, 4–14.2), which was also decreased compared with that in HC, although without statistical significance (P = .15) (figure 1).

Plasma SDF-1 levels and SDF1-3’A genotype. We then analyzed whether there was a relationship between the SDF1-3’A genotype and plasma SDF-1 levels, in uninfected subjects (i.e., the HC and EU groups), in HIV-positive subjects, and in the entire study population. As shown in figure 2, among uninfected subjects there was a clear association between low plasma SDF-1 levels and SDF1-3’A homozygosity. Plasma SDF-1 levels in uninfected SDF1-3’A homozygotes (n = 10) clustered within a narrow range, 4.0–23.2 ng/mL (median, 7.0 ng/mL; IQR, 5.1–16.1). These levels were significantly decreased compared with those found in wild-type SDF1 homozygotes (n = 91) (P = .013) and heterozygotes (n = 47) (P = .029). Plasma SDF-1 concentrations in wild-type homozygotes and heterozygotes were similar (P = .67) and showed a wide range of values, 0.4–198.2 ng/mL (median, 13.4 ng/mL; IQR, 8.2–32.0) in the former and 0.0–170.7 ng/mL (median, 12.4 ng/mL; IQR, 8.85–22.48) in the latter (figure 2).

As in uninfected subjects, among HIV-infected subjects plasma SDF-1 levels in heterozygotes and wild-type homozygotes were similar (P = .29) and showed a wide range of values, whereas among SDF1-3’A homozygotes (n = 6) they were low and clustered within narrow range, 0–48 ng/mL, although these decreased values did not reach statistical significance when compared with either those found in heterozygotes (n = 78; P = .077) or those found in wild-type homozygotes (n = 68; P = .184), likely because of the insufficient number of SDF1-3’A homozygotes in the HIV-positive group. Of the 6 SDF1-3’A homozygote HIV-positive subjects, 2 were LTNP hemophiliacs, and the rest were AS patients.

When the plasma SDF-1 levels of the entire study population (i.e., HC + EU + HIV-positive) were analyzed with respect to SDF1-3’A genotype, the results showed a pattern very similar to that found among uninfected subjects (i.e., the HC + EU group); that is, there were no significant differences between wild-type homozygotes and SDF1-3’A heterozygotes (P = .21), whereas each one of these latter two groups showed very significantly increased plasma SDF-1 concentrations compared with those in SDF1-3’A homozygotes (n = 16) (P ≤ .0047) (figure 2).
CXCR4 expression on T lymphocytes. The proportion of CD4$^+$ and CD8$^+$ T cells expressing surface CXCR4 could be analyzed in the different groups—except in the patients with the most advanced stage of HIV-1 infection (i.e., the AS group), because an insufficient number of cells were available (figure 3). In HC, the median percentages of CXCR4$^+$ CD4$^+$ and CD8$^+$ T cells were 57% (IQR, 41.0%–73.0%) and 50.0% (IQR, 39.0%–65.0%), respectively. Among EU subjects, there was a significant difference between the EU-hem and EU-sex subgroups, in their proportions of CXCR4$^+$ CD4$^+$ and CD8$^+$ T cells, which were significantly elevated in EU-hem compared with EU-sex ($P = .004$ for CXCR4$^+$ CD4$^+$ T cells; $P < .002$ for CXCR4$^+$ CD8$^+$ T cells). In EU-hem, the medians were 72.82% (IQR, 54%–88.58%) and 59.49% (IQR, 39.81%–82.84%) for CXCR4$^+$ CD4$^+$ and CD8$^+$ T cells, respectively, whereas in EU-sex they were 45.1% (IQR, 37.2%–52.7%) and 29.1% (IQR, 22.6%–40.39%), respectively. In addition, the levels of CXCR4$^+$ CD8$^+$ T cells in EU-sex were significantly lower than those in HC ($P < .002$), whereas among EU-hem there was a nonsignificant trend ($P = .10$) toward a higher proportion of CXCR4$^+$ CD4$^+$ T cells, compared with that in HC (figure 3).

Among HIV-infected subjects, the levels of CXCR4$^+$ CD4$^+$ T cells in LTNP (median, 45.1%; IQR, 25.53%–63.45%) did not differ significantly from those in either HC or EU-sex but were clearly lower than those in EU-hem ($P = .001$), whereas in ES patients (median, 66.67%; IQR, 26.06%–78.57%) and IS patients (median, 70.04%; IQR, 49.44%–82.35%), the percentages were higher than those in LTNP, HC, and EU-sex, although these increases were statistically significant only for IS patients compared with LTNP ($P < .005$) and for IS patients compared with EU-sex ($P < .006$). As for the percentages of CXCR4$^+$ CD8$^+$ T cells, in LTNP they were similar to those in EU-sex and were significantly lower than those in HC ($P < .002$) and those in EU-hem ($P < .002$); in ES patients these percentages did not differ from those in HC, LTNP, and EU-sex but were lower than those in EU-hem ($P = .072$; i.e., near the limit of statistical significance); in IS patients, they were significantly higher than those in LTNP ($P = .02$) and also were higher, although without statistical significance ($P = .278$), than those in EU-sex.

The histograms and the mean fluorescence intensity of CXCR4$^+$ cells gated on CD4$^+$ and CD8$^+$ T cells in 5 representative HIV-positive subjects (A–E) with high, intermediate, and low percentage of CXCR4$^+$ cells are shown within each pair of panels A–E; correlation between the 2 parameters, which was observed in entire study population, is depicted in the bottom pair of unlabeled panels. PE, phycoerythrin.

Discussion

This study has shown that SDF1-3'A homozygosity lacks disease progression-retarding effects, because in LTNP the frequency of this genotype is even lower than that in HC and is clearly lower than that in HIV-1–positive progressors. This con-

![Figure 4. Histograms of expression of CXCR4 on gated CD4$^+$ and CD8$^+$ T cells in 5 representative HIV-positive subjects (A–E) with high, intermediate, and low percentage of CXCR4$^+$ cells. Intensity of mean fluorescence (MF) and percentage of CXCR4$^+$ cells are shown within each pair of panels A–E; correlation between the 2 parameters, which was observed in entire study population, is depicted in the bottom pair of unlabeled panels. PE, phycoerythrin.](https://academic.oup.com/jid/article-abstract/186/7/922/865340)
clusion is consistent with those of other studies [31–37], some of which studies had found that SDF1-3'A homozygosity is, in fact, associated with a faster CD4 T cell decrease and a rapid progression to AIDS. The current cross-sectional study is not informative about this possibility, because rapid progressors were not available. It should be mentioned that the SDF1-3'A allele has also been found (1) to abrogate the progression-retarding effect of CCR5Δ32 heterozygosity in children [51], (2) to be associated with an increased risk for non-Hodgkin lymphoma in HIV-positive subjects [52], (3) to increase the likelihood of perinatal infection in mothers but not in their infants [53], and (4) to be present in nonhuman primates most susceptible to lentivirus-induced AIDS and to be absent in those resistant to the latter [54].

Compared with HC, in HIV-1-infected subjects there was a trend toward an increased frequency of SDF1-3'A heterozygosity, although it was significant only for LTNP, a finding not observed in other studies. This discrepancy is unlikely to be attributable to either (1) methodologic inconsistencies, because genotyping was done by the method originally described elsewhere [30] and was repeated in all cases, or (2) an insufficient number of LTNP and/or a nonrigorous definition of this condition, because other studies, with a lower number of LTNP and with a far less strict definition of LTNP condition, have been used as evidence for an association between SDF1-3'A homozygosity and either slow or rapid progression. Perhaps this discrepancy is related to the fact that in our LTNP group, the risk of intravenous drug use predominated (see table 1), whereas in other studies homosexual or bisexual men predominated. None of the LTNP had evidence of non-Hodgkin lymphoma. Because rapid progressors were not available, no conclusions can be drawn about the possible significance, if any, of the increased SDF1-3'A heterozygosity among LTNP.

Among EU subjects, the SDF1-3'A allelic frequency in EU-sex, but not in EU-hem, was significantly underrepresented compared with both that in HIV-positive subjects and that in HC, with absence of mutated homozygotes and a significantly decreased heterozygote frequency. This indicates that SDF1-3'A heterozygosity not only lacks infection-protective effects, as has been suggested elsewhere [30], but also can somehow act as a factor favoring infection through sexual exposure to HIV-1. Such underrepresentation did not occur in EU-hem, a finding that is in accord with the infant SDF1-3'A allele's lack of effect in perinatal transmission [36, 53]. These data suggest that the multiplicity of infection-protecting host factors [14–16, 55] can greatly differ, depending on whether the route of exposure to HIV-1 is sexual or parenteral [56]. This notion may also apply to HIV-1 progression, because the association between delayed progression and an increased frequency of CCR5Δ32 and CR2-64I heterozygosities was found in homosexual men but not in hemophiliacs, intravenous drug users, or children infected perinatally [36, 37, 57, 58]. On the other hand, most (76.8%) of the EU-sex in the present study were women who, both prior to and during the study, engaged in unprotected vaginal sex with HIV-positive men, whereas all EU subjects analyzed by Winkler et al. [30] were homosexual men. This discrepancy between studies might suggest that infection-protecting host factors could differ depending on whether sexual exposure to HIV-1 is anal or vaginal.

The wide range of plasma SDF-1 levels in HC, which also has been observed in a previous study [38], which has used a similar ELISA, suggests that host factors (genetic or epigenetic) that result in phenotypes for high or low SDF-1 production might exist. The plasma-level differences between the different groups are not attributable to blood and plasma collection and storage conditions, because the latter were the same in all cases. Because SDF-1 is the paradigm of homeostatic chemokines, constitutively produced by a broad range of cells except blood leukocytes [7–13], it is conceivable that plasma SDF-1 levels might reflect the overall SDF-1 production of a given host and/or the half-life of this chemokine in this host, events that likely are dependent on host factors (genetic and/or epigenetic). Therefore, we analyzed the relationship between the SDF1-3'A genotype and plasma SDF-1 levels, because it had been proposed that SDF1-3'A homozygosity could result in a higher SDF-1 production [30]. Our data clearly show, for the first time, that, among uninfected subjects, SDF1-3'A homozygosity, but not heterozygosity, is associated with low plasma SDF-1 levels, and the same result was seen in the entire study population. This association might suggest either that SDF1-3'A homozygosity results in decreased SDF-1 production or that there is a linkage between this genotype and other unknown (genetic or epigenetic) host factors that could decrease SDF-1 production and/or promote faster plasma SDF-1 clearance. An in vitro mutation study did not observe differences, in SDF-1 RNA synthesis, between the unmutated and mutated alleles [59]. However, it is unknown whether this system reproduced the physiologic SDF1-3'A homozygous state, and, therefore, experiments with SDF-1–producing cells from mutated and unmutated homozygous healthy subjects are necessary to assess whether there is a relationship between SDF1-3'A genotype and SDF1-3'A production. Whatever the case might be, the association between SDF1-3'A homozygosity and low plasma SDF-1 levels seems congruent with those studies reporting that this genotype is associated with a faster CD4+ T cell decline and AIDS progression [31–35].

Among HIV-positive subjects, plasma SDF-1 levels were significantly increased compared with those in HC, a result seen in LTNP as well as in the ES and IS groups but not in the patients with the most advanced stage of HIV-1 infection (i.e., the AS group). To our knowledge, only 2 studies have investigated plasma SDF-1 in relation to HIV-1 disease progression, and the results are consistent with our findings [38, 39], although neither of those previous studies included LTNP or analyzed the relationship between plasma SDF-1 levels, SDF1-3'A genotype, and CXCR4 expression on T lymphocytes. Our
findings suggest that HIV-1 infection involves overproduction of SDF-1, which might act by delaying progression to AIDS, a notion consistent with recent findings that high plasma SDF-1 levels prevent the emergence of X4 strains [40]. It is noteworthy that such SDF-1 overproduction in HIV-positive patients is not attributable to any kind of chronic viral infection or chronic antigen stimulation, because all EU-hem were hepatitis C virus-positive and had plasma SDF-1 levels similar to those in HC. Further studies, on plasma SDF-1 levels in other infectious and noninfectious chronic inflammatory diseases, are certainly required.

Among the EU-sex and EU-hem groups, there was a reciprocally inverse relationship between plasma SDF-1 levels and expression of surface CXCR4 on CD4+ and CD8+ T lymphocytes; that is, high plasma SDF-1 levels were coupled with low expression of CXCR4 in EU-sex whereas the opposite situation occurred in EU-hem. Although other, unknown immunologic variables might also result in this decreased CXCR4 expression in EU-sex, it is also conceivable that this association reflects an in vivo down-regulation by endogenous SDF-1, as occurs in vitro. In normal subjects, the expression of surface CXCR4 occurs mainly on resting/naive T lymphocytes, whereas the expression of surface CCR5 occurs in memory/activated CD4 T lymphocytes [60]. However, all blood T lymphocytes constitutively express high intracellular stores of CXCR4, which becomes rapidly expressed on the surface of virtually all naive and memory T cells after a very short culture in the absence of SDF-1, whereas surface CXCR4 remains highly down-regulated in the presence of SDF-1 [21, 41, 42]. Other data suggesting an in vivo down-regulation of CXCR4 by endogenous SDF-1 are the findings that (1) surface CXCR4 is selectively down-modulated on intestinal lymphocytes and is coupled with the prominent expression of SDF-1 in mucosal cells [10] and (2) intravenous injection of SDF-1 into mice causes a rapid loss of surface CXCR4 on CD4 T cells [39].

On the other hand, it is obvious that, in EU-sex, the underrepresentation of the SDF1-3′A allele, coupled with high plasma SDF-1 levels and low expression of CXCR4 on CD4+ T lymphocytes, cannot account for this group’s natural resistance to HIV-1 infection, since R5 strains are those which generally transmit the infection, even when X4 strains predominate in the viral inoculum, probably because the mucosa-produced SDF-1 continuously down-modulates CXCR4 on resident HIV target cells [10]. Given (1) the essential role that SDF-1 plays as a homeostatic chemokine governing lymphopoiesis and lymphocyte traffic [6, 23, 61] and (2) SDF-1 production by mucosal dendritic, epithelial, and endothelial cells [10, 11], this association suggests that a phenotype associated with high SDF-1 production is an advantage favoring both optimal maturation of lymphocytes and their migration to vaginal mucosa, which then might be able to “abort” the infection locally. Evidence suggesting a possible immune response-dependent abortion of HIV-1 infection in the vaginal mucosa is available [62, 63].

Among LTNP, high plasma SDF-1 levels were coupled with proportions of CXCR4+ cells that, in CD4+ and CD8+ T cell subsets, respectively, were similar to and lower than those in HC, whereas in patients with advancing HIV-1 infection (i.e., the IS group) (figure 3), the proportions of CXCR4+ cells were significantly increased compared with those in LTNP. These data suggest that high plasma SDF-1 levels coupled with low CXCR4 on T cells is a feature of LTNP and that advancing disease is associated with both enhanced CXCR4 expression on T lymphocytes and a decrease to normal plasma SDF-1 levels in the most advanced stages of HIV-1 infection (i.e., the AS group) (figure 1). This enhanced expression of CXCR4 in advanced disease is in accord with our previously published data [64], as well as with other previously published data, which include reports of an association between enhanced CXCR4 on CD4+ T cells and the emergence of X4 strains [65–67]. It is also congruent with the overexpression of CXCR4 on CD4+ T cells in transgenic mice, which causes severe depletion of peripheral CD4+ T cells [68]. Other studies, however, with fewer patients and without LTNP, have found that the expression of CXCR4 on T cells of HIV-positive patients is decreased compared with that in HC [69–71], although a tendency toward greater activation of CXCR4+ CD4+ T cells was found to occur in patients with the most advanced HIV-1 infection [69]. Because advancing disease involves increased activated/memory T cells, these data might be considered to be consistent with the down-regulation of CXCR4 on CD4+ T cells activated in vitro by CD3 monoclonal antibody [42, 72]. However, it is now known that costimulation with either CD3 CD28 monoclonal antibodies or CD3 plus HIV Tat protein results in the activation and proliferation of CD4+ T cells and causes CXCR4 up-regulation on both resting and activated CD4+ T cells, concomitant with increased infectivity by X4 strains [72]. Given both the essential role that CD28 plays as a costimulatory molecule for antigen-induced activation of T cells and the fact that the highly cytopathic X4 strains mainly replicate in proliferating cells, these findings suggest a relevant mechanism for the progression of HIV-1 disease [72] and are consistent with our findings, in advancing HIV-1 disease, of an increased expression of surface CXCR4 on T cells.

In conclusion, this study has shown the following: (1) SDF1-3′A homozygosity is associated with low plasma SDF-1 levels in uninfected subjects and lacks progression-retarding effects; (2) HIV-1 infection involves SDF-1 overproduction, which is not attributable to any kind of chronic viral infection, because all EU hemophiliacs whom we studied were positive for hepatitis C virus and had normal SDF-1 levels; (3) high plasma SDF-1 levels and low CXCR4 expression on T lymphocytes are a feature of LTNP, whereas in advancing disease the expression of CXCR4 increases and, in the more advanced stages of HIV-1 infection, is accompanied by a decrease of plasma SDF-1; and (4) EU subjects (but not EU hemophiliacs) with sexual, mostly vaginal, exposure to HIV-1 show an underpre-
sentation of SDF1-3′A allele frequency, which is coupled with high plasma SDF-1 levels and low CXCR4 expression.

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References

52. Rabkin CS, Yang Q, Goedert JJ, et al. Chemokine and chemokine receptor
47. Garcia F, Plana M, Vidal C, et al. Dynamics of viral load rebound and
46. Montoro JB, Oliveras J, Lorenzo JI, et al. An association between clotting
42. Bermejo M, Martin-Serrano J, Oberlin E, et al. Activation of blood T lym-
40. Hogan CM, Hammer SM. Host determinants in HIV infection and disease.