Identification of Two Distinct Subsets of Long-Term Nonprogressors with Divergent Viral Activity by Stromal-Derived Factor 1 Chemokine Gene Polymorphism Analysis

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Stromal-derived factor (SDF)–1, the natural ligand for CXCR4, is present in a common polymorphic variant defined by a G→A transition in the 3′ untranslated region of the gene. In persons infected with human immunodeficiency virus type 1 (HIV-1), the homozygous genotype (*SDF1*-3′A/3′A) has been postulated to interfere with the appearance of T-tropic syncytium-inducing strains. The polymorphism of *SDF1* was correlated with HIV-1 phenotype, plasma viremia, and unspliced and multiply spliced specific transcripts in 158 virologically characterized HIV-1–infected patients (39 recent seroconverters, 75 typical progressors, and 44 AIDS patients) and in 42 HIV-1–infected long-term nonprogressors (LTNPs). Analysis of *SDF1* allele distribution revealed that *SDF1*-3′A/3′A status is associated with low CD4 cell count ($P = .0449$) but not with a specific HIV-1 phenotype. In LTNPs, *SDF1*-+/+ condition defined a subset of persons with lower HIV-1 replication than in heterozygous subjects. The low viral activity in *SDF1*-+/+ LTNPs suggests that other factors play a major role in vivo in determining the course of HIV-1 infection.

An important advance in AIDS research is the demonstration that human immunodeficiency virus type 1 (HIV-1) uses families of both α and β chemokine receptors as coreceptors for entry into CD4 target cells [1]. These findings prompted the research for polymorphism in chemokine receptor genes in HIV-1–infected persons to assess the role of these molecules in determining the course of infection [2–4]. Because, in most studies, the prevalence of *CCR5*-Δ32 heterozygosity is reduced in AIDS patients, it has been suggested that the *CCR5*-Δ32 heterozygous condition is associated with nonprogression [2, 5, 6]. However, it has not been excluded that a combination of several allelic forms of CCR genes may play a role in HIV-1 disease progression [3, 7].

Stromal-derived factor (SDF)–1 is the natural ligand for CXCR4 [8]. Recently, a common polymorphic variant defined by a G→A transition in the 3′ untranslated region (UTR) of the *SDF1* gene was investigated in five large cohorts. The results suggested that the homozygous genotype (*SDF1*-3′A/3′A) is associated with a slow progression to AIDS in HIV-1–infected persons [9]. In that cross-sectional study, a gradation of the 3′A/3′A genotype prevalence across increasingly severe AIDS end points was observed, leading to the hypothesis that the homozygous status may interfere with the appearance of T-tropic syncytium-inducing (SI) HIV-1 strains. The mechanism could involve the up-regulation of SDF1 protein, which competes with HIV-1 to bind the CXCR4 receptor. However, a recent retrospective study failed to identify a correlation between the *SDF1* homozygous condition and the total frequency of SI HIV-1 variants, although the prolonged survival after AIDS diagnosis was confirmed in a population of homosexual HIV-1–positive patients [10]. A subsequent study suggested that progression to death, but not the clinical end point of AIDS, is related to *SDF1*-3′A allelic status [11]. A clear correlation of *SDF1*-3′A homozygosity with protection against disease progression was questioned in two other recent reports [12, 13].

Although additional evidence on the interaction between HIV-1 and α and β chemokine receptors was provided by recent crystallographic studies [14], no conclusive data are available regarding the relationship between *SDF1* genotype and levels of viral replication in HIV-1–infected persons with typical or

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long-term nonprogressive disease courses. In this study, we addressed the role of *SDF1* polymorphism in a virologically characterized HIV-1–positive population and evaluated the relationship between *SDF1* status and the degree of immune depletion and non-SI (NSI) or SI HIV-1 phenotype. In addition, the relationship among HIV-1 replication, phenotype, and *SDF1* genotype was analyzed within a group of highly selected long-term nonprogressors (LTNPs).

Patients and Methods

Donors and clinical samples. Samples from 105 healthy white controls living in the Milan area of Italy (80 men and 25 women), median age 32 years (range, 25–45), were obtained from the Transfusion Service, Department of Immuno-Hematology, L. Sacco Hospital, Milan. Eighteen female partners repeatedly exposed to HIV-1 were defined as high-risk persons. In total, 158 HIV-1–infected outpatients of the Institute of Infectious and Tropical Diseases, University of Milan, were consecutively enrolled during 1994–1997. These were classified in three groups: (1) recent seroconverters (29 men and 10 women, median age 34 years [range, 22–46], with documented seroconversion within 12 months before the study); (2) typical progressors (59 men and 16 women, median age 35 years [range, 21–47], with HIV-1 antibodies first detected during 1988–1994, declining CD4 cell slopes, overall CD4 cell loss >30%, and <500 CD4 cells/μL at enrollment); and (3) 38 men and 6 women with AIDS, according to the 1987 CDC definition (median age 32 years [range, 21–43]). Median CD4 cell counts per microliter by group were 537 (range, 317–1735), 230 (range, 1–481), and 46 (range, 1–209), respectively. All subjects, except those with AIDS, were naive for antiretroviral therapy. Risk factors for HIV-1 infection were homosexual contacts (n = 30, 35, and 23), intravenous drug use (IVDU; n = 6, 40, and 21), and heterosexual sex (n = 3, 0, and 0) in recent seroconverters, typical progressors, and AIDS patients, respectively. Forty-two subjects (30 men and 12 women, median age 35 years [range, 28–68]) in the Resistant Host Perspective Study were defined as LTNPs. Risk factors in this group were homosexual contacts (n = 6) and IVDU (n = 36). These subjects represent a group of untreated, asymptomatic persons with a median infection duration of 12 years (range, 10–16) and a CD4 T cell count persistently >500 cells/μL in a median of 14 consecutive determinations. They had a median of 725 cells/μL (range, 502–1585) at enrollment in the cohort.

SDF1 *polymerase chain reaction (PCR) restriction fragment length polymorphism.* Amplification of the 3′ UTR region of the *SDF1* gene was done by PCR with primers 5′-CAGTCA-ACCTGGGCAAAGCC-3′ and 5′-AGCTTTGGTCCTGAGAG-TCC-3′ (nt 779–798 and 1061–1080 of the reference sequence; GenBank accession number L36033) in a 50-μL final volume, as described [9]. The reaction mixture contained 200 ng of DNA, 0.2 m*M* dNTPs, 2 m*M* $MgCl_2$, 20 pmol of each primer, and 2 U of *Taq* polymerase (Perkin-Elmer, Norwalk, CT). Thirty-five cycles were done as follows: 30 s at 94°C, 30 s at 60°C, and 2 min at 72°C, with a final extension of 10 min at 72°C. Cleavage of PCR products was done by using 5 U of *Msp*I restriction endonuclease (Sigma, St. Louis) in 20 μL, followed by incubation at 37°C for 1 h. The cleavage products and appropriate controls were analyzed by 2% agarose gel electrophoresis. Genotypic analysis compared both undigested and digested patient samples. *SDF1*-+/+, *SDF1*-+/3′A, and *SDF1*-3′A/3′A genotypes were detected as distinct bands, as follows: 2 bands of 202 and 100 bp; 3 bands of 302, 202, and 100 bp; and a single band of 302 bp, respectively.

HIV-1 isolation and phenotype evaluation. Peripheral blood mononuclear cells (PBMC) from HIV-1–infected subjects were isolated by standard gradient-based procedures. HIV-1 was isolated by cocultivation [15]. HIV-1 p24 antigen was assayed in culture supernatants by commercial ELISA (DuPont, Wilmington, DE). Virus phenotype was tested in an MT-2 cell line as described [16].

HIV-1 plasma viremia and specific transcript detection. The HIV-1 cell-free RNA present in plasma samples and HIV-1 cell-associated RNA, both unspliced and multiply spliced transcripts, were quantified by competitive reverse transcription–PCR procedures as described elsewhere [17].

Statistical analysis. Genetic and virologic data were analyzed by χ^2 test with Yates's correction, and Fisher's exact test was used to compare proportions between groups. Quantitative molecular indices of viral activity and CD4 cell values were compared by Mann-Whitney or Kruskal Wallis tests. The analyses were done with computer software (StatView version 4.5; Abacus Concepts, Berkeley, CA).

Results

SDF1 *gene polymorphism in subgroups of HIV-1–infected subjects.* Table 1 shows the results of *SDF1* allele analysis in 123 HIV-1–negative subjects and in 158 consecutively enrolled HIV-

Table 1. *SDF1* polymorphism in study population.

Group	*SDF1* genotype[a]			Allelic frequency
	+/+	+/3′A	3′A/3′A	
Healthy controls (n=105)	62 (59.0)	40 (38.1)	3 (2.9)	0.219
High-risk seronegative (n=18)	11 (61.1)	6 (33.3)	1 (5.6)	0.222
Recent seroconverters (n=39)	25 (64.1)	12 (30.8)	2 (5.1)	0.205
Typical progressors (n=75)	45 (60)	26 (34.7)	4 (5.3)	0.227
AIDS progressors (n=44)	26 (59.1)	13 (29.5)	5 (11.4)	0.261
Long-term nonprogressors (n=42)	26 (61.9)	15 (35.7)	1 (2.4)	0.202

NOTE. *SDF1,* stromal-derived factor 1 gene. Values are no. (%) unless otherwise indicated.

[a] *SDF1* genotypes: +/+, +/3′A, and 3′A/3′A are wild type, homozygous, and heterozygous genotypes, respectively.

Table 2. CD4 cell counts and human immunodeficiency virus type 1 (HIV-1) phenotype according to *SDF1* polymorphism in a consecutive series of 158 HIV-1–positive patients.

SDF1 polymorphism	CD4 cells/μL, median (range)	HIV-1 phenotype		
		UND	NSI	SI
+/+ (*n* = 96)	210 (1–1326)	5 (5.2%)	65 (67.7%)	26 (27.1%)
+/3′A (*n* = 51)	209 (1–1735)	3 (5.9%)	38 (74.5%)	10 (19.6%)
3′A/3′A (*n* = 11)	56 (1–344)	0	9 (81.8%)	2 (18.2%)
P	.0449[a]		>.05[b]	

NOTE. *SDF1,* stromal-derived factor 1 gene; UND, undetermined phenotype in isolation-negative subjects; NSI, non–syncytia-inducing strains; SI, syncytia-inducing strains.

[a] Kruskal Wallis test.

[b] χ^2 test.

1–positive persons (39 recent seroconverters, 75 typical progressors, and 44 AIDS patients) and 42 LTNPs. A comparable distribution of each genotype and similar allelic frequency were observed in the different groups, and no difference in prevalence of *SDF1* polymorphism was seen in 123 HIV-1–negative subjects, compared with the 158 HIV-1–infected patients (59.3% vs. 60.8%, 37.4% vs. 32.3%, and 3.3% vs. 7% of *SDF1*-+/+, *SDF1*-+/3′A, and *SDF1*-3′A/3′A status, respectively). Similarly, the percentages of allele distribution did not differ within HIV-1–negative or –positive subjects (controls vs. high-risk persons).

Among the consecutively enrolled HIV-1–positive subjects, those with AIDS had the highest frequency of *SDF1*-3′A/3′A genes (11.4%); however, this prevalence was not significantly different from that of recent seroconverters or typical progressors. A similar frequency of *SDF1* polymorphism was observed in LTNPs. The *SDF1* genotypic status was correlated with immune damage, as defined by absolute number of CD4 cells, and the prevalence of virus variants associated with the progression of HIV-1 disease, as reflected by HIV-1 phenotype. As shown in table 2, *SDF1*-3′A/3′A homozygous status was associated with the lowest CD4 cell count (median, 56/μL; range, 1–344). There was a weak, albeit significant, difference in CD4 cell numbers among HIV-1–seropositive subjects in the consecutive series (recent seroconverters, typical progressors, and AIDS patients) stratified by *SDF1* genotype ($P = .0449$). In contrast, no difference was observed among subjects when considering HIV-1 phenotype. The proportion of subjects harboring NSI isolates was similar for each allele status. Of note, 2 subjects with the homozygous mutation of *SDF1* harbored SI variants, suggesting that these patients were not protected from the NSI→SI shift in the course of HIV-1 infection.

SDF1 *polymorphism and HIV-1 activity in LTNPs.* Figure 1 illustrates the degree of replication, frequency of virus isolation, and number of CD4 cells in LTNPs stratified by *SDF1*-+/+ and *SDF1*-+/3′A status. As expected, subjects with *SDF1* wild type did not differ from those with heterozygous genotype in CD4 cell counts. Of interest, persons with *SDF1*-+/+ showed lower indices of viral activity than those with *SDF1*-+/3′A. Median HIV-1 RNA per milliliter of plasma and unspliced and multiply spliced specific transcripts per 2×10^5 PBMC were 260 (range, 10–5470), 3 (range, 0–52), and 0 (range, 0–1) and 2019 (range, 100–19,193), 9 (range, 1–218), and 0 (range, 0–22) in *SDF1* wild type and heterozygous subjects, respectively. All between-group comparisons were statistically significant ($P = .0021$, .016, and .0031 for plasma viremia and unspliced and multiply spliced transcripts, respectively). One LTNP (2.3%)

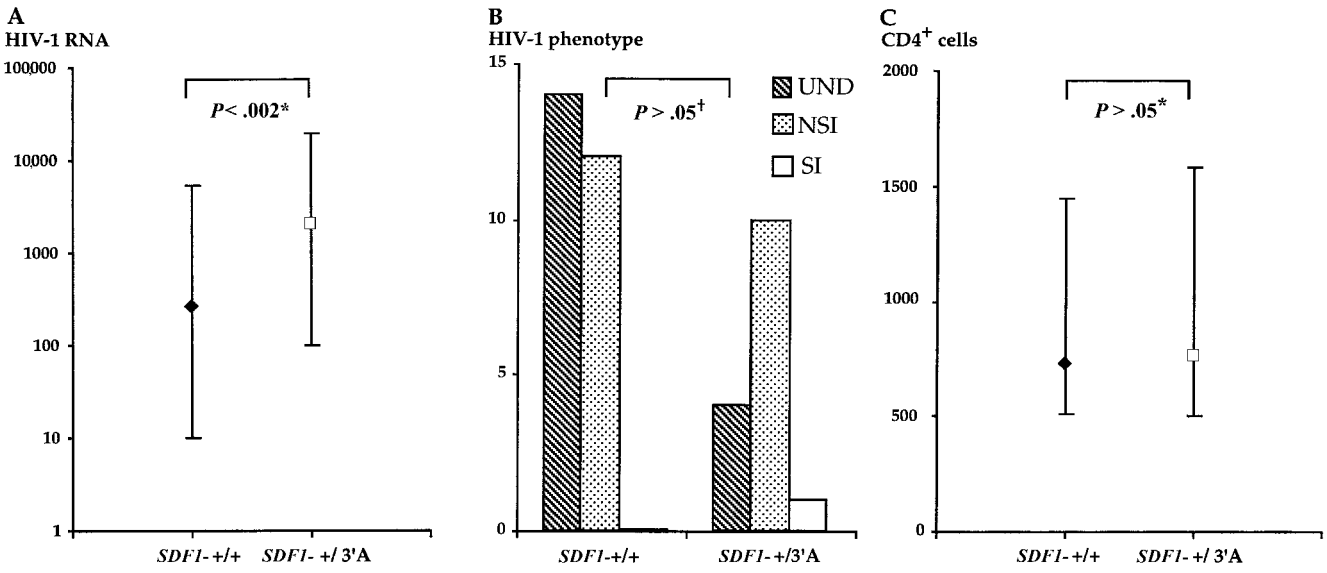

Figure 1. *A,* Human immunodeficiency virus type 1 (HIV-1) RNA copies/mL. *B,* Proportion of subjects with HIV-1 isolates with different phenotypes (UND, undetermined phenotype in isolation-negative subjects; NSI, non–syncytium-inducing strains; SI, syncytium-inducing strains). *C,* CD4 cells/μL in *SDF1*-+/+ and *SDF1*-+/3′A long-term nonprogressors. * Mann-Whitney test; † χ^2 test.

was homozygous for the *SDF1*-3′A allele; in this subject, HIV-1 plasma viremia and unspliced and multiply spliced transcripts in PBMC were 836 copies/mL and 10 and 1 per 2×10^5 PBMC, respectively. The *P* values were not different after the inclusion of the homozygous *SDF1*-3′A/3′A subject in the analysis (Kruskal Wallis test, $P = .008$, .0416, and .004 for HIV-1 cell-free RNA and unspliced and multiply spliced intracellular transcripts, respectively). Fourteen (77.7%) of 18 HIV-negative subjects were *SDF1*-+/+; the proportion of NSI isolates was not different in *SDF1*-+/+ and *SDF1*-+/3′A subjects. Median age (35 vs. 35 years), sex (18 men and 8 women vs. 11 men and 4 women), risk groups (24 IVDUs and 2 homosexual contacts vs. 11 IVDUs and 4 homosexual contacts), and median length of infection (12 vs. 11 months) were similar in *SDF1* wild type and heterozygous LTNPs.

Discussion

In the last few years, numerous in vivo molecular studies have indicated that progression to AIDS is strongly associated with high levels of HIV-1 replication [18, 19]. In this context, studies that aim to address the correlation between virus load and the presence of several polymorphisms in HIV-1 coreceptor genes in vivo are of primary importance. Persons with long-term nonprogressive courses of HIV-1 infection represent a special subset of subjects and enable the study of the complex interaction between host and viral determinants influencing the rate of progression and affecting the time-dependent prognosis of the disease. In these subjects, host factors, including genetic status and immunologic responses, have been linked to very slow progression of HIV-1 disease [12, 13, 20, 21].

In addition to *CCR5*-Δ32 and *CCR2*-64I polymorphisms, it has been postulated that *SDF1* polymorphism may assist with a favorable course of HIV-1 disease. SDF1α may interfere with T-tropic SI HIV-1 strains by binding and down-regulating the CXCR4 receptor [8, 22, 23]. It is conceivable that excessive amounts of full-length SDF1α may occupy the HIV-1 CXCR4 coreceptor and inhibit virus entry. However, no direct evidence is available at present to support this hypothesis. Moreover, little is known about the mechanism of action of the isoform SDF1β, and SDF1β interference in CXCR4 binding of HIV-1 has not been shown. Although the high conservation between human and murine SDF1β 3′UTRs [24] highlights the importance of this segment in influencing posttranscriptional processing of SDF1β mRNAs, SDF1α and SDF1β 3′UTRs differ in sequence, and no similarity in the possible function of the UTRs has been established.

To address the role of *SDF1* polymorphism, we analyzed the frequency of *SDF1* polymorphism in a population of HIV-1–infected patients representing all stages of the disease and in a group of highly selected LTNPs. The data indicate that *SDF1*-3′A/3′A is associated with low CD4 cell counts, and neither persons with *SDF1*-3′A/3′A nor those with *SDF1*-+/3′A are protected against emergence of SI variants. Two subsets of LTNPs with distinct patterns of viral activity were identified: the molecular parameters of HIV-1 replication were significantly lower in persons with *SDF1*-+/+ than in those with *SDF1*-+/3′A and those with *SDF1*-3′A/3′A. Our data are in agreement with the results of recent studies that considered the influence of *SDF1* allelic status and the outcome of HIV-1 disease in subjects with a very slow disease progression compared with that of typical progressors [12, 13]. In particular, the prevalence of *SDF1*-3′A/3′A and *SDF1*-+/3′A allelic conditions observed in our study within LTNPs was similar to that observed by Magierowska et al. [12]. Moreover, Hendel et al. [13] found no significant difference in virus load among slow progressors stratified by *SDF1* polymorphism.

The NSI→SI shift has been recognized as a defined pathogenic event during HIV-1 disease [25]. It has been suggested that up-regulation of SDF1α production in *SDF1*-3′A/3′A and *SDF1*-+/3′A patients may prevent the appearance of SI strains commonly observed in the late phase of HIV-1 infection [9]. However, there has been no direct evidence to demonstrate the absence of SI strains in persons with *SDF1*-3′A/3′A. New insights into HIV-1 coreceptor use recently led to a new classification for HIV-1 strains. Both previous and present findings concerning HIV-1 cytopathicity and tropism and coreceptor use may more accurately represent the physiologic behavior of HIV-1 in the infected host [26]. It is possible that HIV-1 tropism, defined by the use of *CCR5*- and CXCR4-expressing cell lines, may explain our results.

The data from LTNPs shown here deserve specific comment. First, the protection conferred by the *SDF1*-3′A allele is not required for long-term nonprogression, as indicated by the high percentage of LTNPs with the *SDF1*-+/+ genotype. Second, the presence of the 3′A mutation identifies a subset of LTNPs characterized by significantly more active HIV-1 replication, even if low to moderate. Because there are conflicting data regarding the role of the *SDF1* homozygous genotype [9–13], further studies are needed to evaluate whether the immune-mediated mechanisms involved in controlling viral replication in *SDF1*-+/3′A LTNPs are different, on a qualitative or quantitative basis, from those in LTNPs without this 3′A mutation.

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