



## SDF-1 gene polymorphisms and syncytia induction in Brazilian HIV-1 infected individuals

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### Abstract

Stromal-derived factor (SDF-1) is the principal ligand for CXCR4, a co-receptor with CD4 for T lymphocyte cell line-tropic human immunodeficiency virus-type 1 (HIV-1). A common polymorphism, SDF1-3' A, was identified in an evolutionary conserved segment of the 3' untranslated region of the SDF-1 gene. Sequence analysis revealed a common variant at position 801, a G → A transition referred to as SDF1-3' A. Because this variant eliminates the Msp I restriction site PCR-restriction fragment length polymorphism (RFLP) analysis was used for rapid detection of genotypes. We genotyped 62 HIV infected patients and 60 non-HIV blood donors by RFLP analysis. We also assessed syncytia formation through co-culture of MT-2 cells with peripheral blood mononuclear cells from HIV patients. Syncytium-inducing HIV-1 variants have been shown to be clinically significant in the pathogenesis of HIV-1 infection. In our study, we detected a low frequency of 3'A/3'A (5%) in the blood donors but this genotype was absent in all HIV patients. We found that 41 (68%) HIV patients including syncytia inducing (SI) and non-syncytia inducing (NSI) groups contained the wild type (wt/wt) genotype for SDF-1. Our data indicate that there is no correlation between SDF-1 alleles and syncytium inducing HIV.

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### 1. Introduction

A polymorphism in an evolutionary conserved segment of the 3' untranslated region (3' UTR) of the SDF-1 gene (SDF-3'A) has been described. HIV infected patients homozygous for this mutation exhibited a significantly delayed progression to AIDS and an even more significant decrease in mortality [1]. It has been known that the SDF1-3'A mutation could result in increased SDF-1 production, resulting in late infection due to the strong competition with syncytia inducing (SI) variants at the CXCR-4 chemokine receptor level. Recently, it was shown that patients

homozygous for the SDF-1 3'A variant had more-rapid disease progression [2]. Therefore, in this study we examined the distribution of the SDF-1 allele in HIV infected Brazilian patients and representative blood donors of European, African and Asiatic ancestry.

T-tropic SI viruses generally appear late in the course of infection during the so-called 'phenotypic switch' that often precedes the onset of AIDS symptoms [3,4].

Viral tropism is determined by the ability of the virus to bind both CD4 and the specific chemokine co-receptor. CXCR4 is the chemokine receptor most commonly used by T-cell-tropic HIV-1 [5]. T-tropic HIV-1 enters target cells by means of CD4 and CXCR4 binding as a co-receptor complex, although in some cases T-tropic strains can also use the CCR5 co-receptor [6]. Stromal-derived factor (SDF-1, also named pre-B cell growth stimulating factor) is a potent chemotactic cytokine and the cellular ligand for

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CXCR4. Recent experiments have shown that SDF-1 $\alpha$  (one of two splice variants of the SDF1 gene) is capable of down-regulating CXCR-4 on cells by endocytosis, effectively blocking infection by T-tropic but not M-tropic HIV-1 strains [5].

The use of available CXCR4 co-receptors by viral strains that emerge during late stage HIV-1 infection, together with the demonstration that SDF-1 effectively inhibits HIV-1 replication, prompted a polymorphism search for SDF-1 structural gene variants that might influence HIV-1 transmission or pathogenesis [1].

Sequence analysis of the SDF gene (GenBank accession number L36033) revealed a common variant at position 801 (counting from the ATG start codon), a G  $\rightarrow$  A transition in the 3' untranslated region (3' UTR). This newly identified polymorphism designated SDF1-3'A is present in the SDF-1 $\beta$  but not the SDF-1 $\alpha$  transcript. Because this variant eliminates the Msp I restriction site, PCR-restriction fragment length polymorphism (RFLP) analysis was used for rapid detection of genotypes. This variant allele of the SDF1 gene may have an important regulatory function, by increasing the production of SDF-1 which, when bound to CXCR4 prevents the virus from entering and infecting T cells [1,7].

We genotyped 62 HIV infected patients from the Clinical Hospital (University of São Paulo) and 60 non-HIV blood donors from the Regional Blood Center of Ribeirão Preto (São Paulo) by RFLP using the Msp I restriction enzyme. We also analyzed syncytia induction of peripheral blood mononuclear cells (PBMC) from HIV patients by co-culture with MT-2 cells.

## 2. Results

All the HIV patients selected for our study had stable CD4 cell counts above  $200 \times 10^6/L$ .

As shown in Table 1, the non-syncytia inducing (NSI) phenotype was present in 21.6% (13/62) in which 70% (9/13) were wt/wt genotype and 30% (4/13) were 3'A/wt. We did not detect 3'A/3'A genotype in either the SI or NSI HIV patients.

We also analyzed 60 non-HIV blood donors from the Blood Center of Ribeirão Preto and verified a low frequency of SDF1-3'A/3'A (5%) carriers among these healthy donors. The heterozygous 3'A/wt was detected in the e 18 (30%) individuals and homozygous wt/wt in 39 (65%).

Table 1  
Analysis for development of syncytia in the 62 HIV patients. Stromal cell-derived factor (SDF1) genotype using Msp-I restriction enzyme

HIV patients	SDF-RFLP wt/wt ( $n = 41$ )	SDF-RFLP 3'A/wt ( $n = 21$ )
NSI ( $n = 13$ )	9 (70%)	4 (30%)
SI ( $n = 49$ )	32 (65%)	17 (34%)

## 3. Discussion and conclusions

It has been demonstrated that T-tropic HIV-1 env proteins can directly interact with CXCR4. In this context T cell tropic strains require the expression of CXCR4 in conjunction with CD4 for membrane fusion and infection to occur [8]. These G-protein-coupled seven-transmembrane receptors have been identified as co-receptors for HIV-1.

Syncytium-inducing HIV-1 variants have been shown to be clinically significant in the pathogenesis of HIV-1 infection [9,10]. The ability of HIV-1 isolates to produce cytopathic effects in a human T-cell leukemia virus type I-transformed lymphoblastoid cell line (MT-2 cells) has been shown to be sensitive to and specific for syncytium-inducing capacity [11,12]. While syncytium-inducing viruses have been detected at all stages of HIV-1 infection, they are more commonly found among individuals with advanced disease [3].

The syncytium-inducing phenotype is only one of many traits along a continuum of HIV-1 phenotypes. The SI phenotype is usually related to rapid replication rates to high titers and the ability to infect transformed T-cell lines. However all these characteristics can be conferred by the same determinants that produce the SI phenotype.

The rapid evolution of HIV-1 in infected individuals is due to high replication rates, error-prone replication and selective pressure exerted by the host's immune system [13]. Several different types of variants, which can be distinguished by their in vitro phenotypes, can emerge over the course of infection and disease progression [14]. Syncytium-inducing HIV-1 variants have been shown to be clinically significant in the pathogenesis of HIV-1 infection. It is known that absence of SDF1-3'A/3'A genotype may indicate rapid progression to AIDS which may be involved in the down regulation of SDF-1.

Delayed disease progression in HIV-1 infection has been previously shown to be associated with homozygosity for the G  $\rightarrow$  A transition in the 3'UTR of the SDF1 gene [1]. Contrary to this observation, van Rij et al. [15] have described a more rapid progression to AIDS at a higher CD4<sup>+</sup> T-cell count in individuals with SDF1-3'A/3'A genotype, but subsequently followed by a prolonged survival time after AIDS diagnosis.

While syncytium-inducing viruses have been detected at all stages of HIV-1 infection, they are more commonly found among individuals with advanced disease.

All the patients in our study, both SI and NSI demonstrated absence of 3'A/3'A genotype suggesting progression to AIDS. Furthermore, we did not detect the SDF1-3'A/3'A genotype in the long-term survivor group (data not shown) and the wild type homozygous genotype was observed in 68% of HIV patients. Our results demonstrate no correlation between SDF1-3'A genotype and syncytium inducing HIV. The genetic background involving SDF-1 alleles related to HIV pathogenesis remains to be clarified in HIV-1 infected patients.

## 4. Subjects and methods

Following approval from the Human Ethics Committee of the University of São Paulo, blood samples were obtained from patients and blood donors.

### 4.1. Study population

We studied 62 HIV<sup>+</sup> Brazilian patients, from the Clinical Hospital—University of São Paulo. All clinical stages of AIDS (asymptomatic, symptomatic and AIDS) were presented, according to the 1993 Center for disease Control and Prevention (CDC) definition. We also studied 60 non-HIV blood donors the Regional Blood Center of Ribeirão Preto.

### 4.2. Polymerase chain reaction

Genomic DNA was isolated from peripheral blood cells using ‘Super Quick-Gene DNA Isolation Kit’—Analytical Genetic Testing Center (AGTC) and 100 ng of DNA was analysed by PCR with primers for SDF 3′UTR-F (sense, 5′-CAGTCAACCTGGGCAAAGCC-3′) and SDF 3′UTR-R2 (antisense, 5′-CCTGAGAGTCCTTTTGC GGG-3′). The G → A transition in SDF1-3′A alleles eliminates a MspI site allowing the use of a PCR restriction fragment length polymorphism assay for rapid detection of SDF-1 genotypes [1]. Samples were amplified with taq polymerase (Pharmacia Biotech, São Paulo, SP, Brazil) in the buffer provided, with a final concentration of 1.5 mmol/l. Conditions of PCR comprised 5 min denaturation at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C and 10 min elongation at 72 °C in a Perkin Elmer thermocycler. PCR products of 293 base pairs were analysed by electrophoresis in a 2% agarose gel and visualized by means of UV fluorescence after staining with ethidium bromide.

### 4.3. SDF-1 genotyping

PCR products were subjected to restriction analysis with MspI for 3 h at 37 °C (Gibco, BRL, Maryland, USA) and analyzed by electrophoresis in a 2% agarose gel, yielding a 100 and 193 base-pair product in the case of a SDF-1 wild-type allele (SDF1-wt) and a 293 base-pair product in the case of SDF1-3′A.

#### 4.3.1. Syncytium-Inducing phenotypes detection assay

Human PBMC obtained from heparinized blood from HIV<sup>+</sup> patients from the Clinical Hospital of University of São Paulo, Ribeirão Preto—São Paulo were separated on Ficoll-Hypaque (Sigma, St Louis, MO), maintained in RPMI 1640 medium (Life Technologies Inc., São Paulo, SP, Brazil) and supplemented with 10% fetal bovine serum (FBS: Life Technologies Inc.), 2 mM L-glutamine (Life Technologies Inc.) and penicillin-streptomycin (100 units/ml; Sigma). Human T-cell line MT-2 derived from human T-cell leukemia cells isolated from cord blood

lymphocytes and co-cultured with cells from patients with adult T-cell leukemia was provided by *NIH AIDS Research and Reference Reagent Program* operated by Mckesson Bioservices. MT-2 cells ( $3 \times 10^4$  cells) were co-cultured with PBMC ( $3 \times 10^5$  cells) from HIV-1 infected patients in triplicate wells of flat-bottomed 96-well microtiter plates in a final volume of 200  $\mu$ l per well. Plates were incubated at 37 °C with 5% CO<sub>2</sub>. Cultures were examined every 3 days for syncytium formation using an inverted microscope until termination (14–28 days depending on the experiment). Syncytium formation was defined as six to ten multinucleated giant cells. In the seventh day of syncytium positivity was noted. Following inspection, cultures were split by removing 100  $\mu$ l of fresh medium from each well. If no syncytia had been observed by the day of assay termination, the isolate was registered as (NSI).

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