AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 17, Number 13, 2001, pp. 1241–1247
Mary Ann Liebert, Inc.

Biological Characterization and Chemokine Receptor Usage of HIV Type 1 Isolates Prevalent in Brazil

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ABSTRACT

The human immunodeficiency virus type 1 (HIV-1), the etiological agent of the acquired immunodeficiency syndrome (AIDS), shows a variety of biological properties, which may constitute an obstacle to development of effective vaccines or antiretroviral therapy. To characterize Brazilian strains of HIV-1, we studied 24 viruses isolated from blood samples of HIV-1-positive patients from different regions of the country. To examine the cell tropism and the virus ability to form syncytia, primary macrophages and the $CD4^+$ T cell line MT-2 were infected with these viruses. We found that 22 isolates replicated well in macrophages (macrophage-tropic isolates), 2 infected only MT-2 cells (T cell line tropic variants), while 6 of them grew in both cells. We found 8 syncytium-inducing (SI) and 16 non-SI (NSI) isolates. Continuous cultures of 18 isolates were established in the $CCR5^+/CXCR4^+$ cell line PM-1, and SI/NSI features of these viruses were confirmed by cell fusion assay with uninfected $CD4^+$ T cell lines (PM-1, MT-2, H9, and SUP-T1). The coreceptor usage of 18 isolates was investigated by infecting U87 cells transfected with CD4 and chemokine receptors, and we found that 11 isolates infected only $CCR5^+$ cells, 3 only $CXCR4^+$ cells, whereas 4 used both coreceptors. We also observed that X4 isolates were more sensitive to neutralization by dextran sulfate than R5 or R5X4 viruses. Our findings show that the Brazilian isolates are phenotypically similar to those prevalent in other regions, which could mean that therapeutic strategies based on HIV-1 phenotypic properties would be efficient in Brazil, as in other countries.

INTRODUCTION

THE BIOLOGICAL AND GENETIC DIVERSITY of the human immunodeficiency virus type 1 (HIV-1), the etiological agent of the acquired immunodeficiency syndrome (AIDS), may constitute an obstacle to the development of effective vaccines or antiretroviral agents. In fact, the viral polymorphism[1] is associated with the emergence of variants resistant to the anti-HIV-1 drugs presently in use,[2,3] as well as with the failure of potential vaccines to inhibit the infection mediated by primary isolates.[4,5] Since the detection of distinct patterns of HIV replication *in vitro*,[6] different HIV-1 phenotypes have been identified according to virus properties in cell culture. In general, HIV-1 isolates that poorly replicate in monocyte-derived macrophages (MDM) and infect and induce syncytium formation in $CD4^+$ T cell lines (such as MT-2 and H9) are referred to as T cell line tropic, syncytium-inducing (SI) viruses.[7,8] The detection of SI isolates in clinical samples has been associated with declines in $CD4^+$ T cell numbers, increases in viral load, and progression from the asymptomatic clinical status to AIDS.[7,9] On the other hand, isolates that productively infect MDM and do not infect or form syncytia in tumor cell lines are often designated macrophage-tropic or non-syncytium-inducing (NSI) viruses.[9] Macrophage-tropic and NSI isolates have been associated with the asymptomatic clinical status of the HIV-1-infected patients, and are the phenotypes preferentially transmitted *in vivo*.[7,10,11] All HIV-1 isolates have the ability to infect activated peripheral blood mononuclear cells (PBMC).

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Regarding sensitivity to humoral immune response, tissue culture laboratory-adapted (TCLA) SI viruses are commonly neutralized by antibodies,[12–14] whereas clinical (primary) isolates are markedly resistant to anti-HIV-1 hyperimmune sera (12). Lately, the HIV-1 phenotypes have also been identified based on the chemokine receptor to which they bind on the target cell membrane, simultaneously with the CD4 molecule, during the infection process. Accordingly, viruses that preferentially interact with the β-chemokine receptor CCR5 as a coreceptor are termed R5-tropic isolates, those that bind to the α-chemokine receptor CXCR4 are termed X4-tropic, and the viruses able to use either one are classified as R5X4 viruses dual-tropic.[8] Several authors demonstrated a close correlation between HIV-1 phenotype and the molecular structure of the third variable domain (V3 loop) of the virus envelope protein (gp120/41). Indeed, mutations in this region are associated with changes in several biological properties, such as chemokine receptor usage, cell tropism, sensitivity to neutralizing antibodies, and virulence.[7,15–23]

HIV-1 subtype B predominates in Brazil, but an increasing number of non-B infections has been detected lately.[24] Interestingly, almost 40% of the Brazilian subtype B present a GWGR motif at the top of the V3 loop (subtype B″).[25] Although the genetic polymorphism of Brazilian HIV-1 isolates is well known, their phenotype has been only partially investigated.[24] To contribute to a better understanding of the biological properties of the HIV-1 strains circulating in Brazil, we studied several *in vitro* phenotype features of HIV-1 primary viruses, which were isolated from blood samples of HIV-1-positive individuals living in different cities of this country. In this report we show, based on results obtained with infection of MT-2 cells, induction of syncytium formation, tropism to human macrophages, chemokine receptor usage, and the sensitivity to dextran sulfate, that the isolates of HIV-1 prevalent in Brazil present biological properties similar to those described for HIV-1 isolates from other countries.

MATERIALS AND METHODS

Virus isolates

We selected for this study a panel of 24 HIV-1 primary isolates that are part of the repository of the Brazilian Network for HIV Isolation and Characterization.[26] The viruses were isolated from 7 HIV-1-positive patients living in the city of Santos (State of São Paulo), 12 in Rio de Janeiro (State of Rio de Janeiro), 4 in Belo Horizonte (State of Minas Gerais), and one in Salvador (State of Bahia). The virus isolation and the genetic, immunological, and preliminary biological characterization of the majority of these viruses, as well as the epidemiological, clinical, and laboratory data of most of the patients, were recently published.[24] Four isolates (96BRRJ026, 97BRRJ040, 97BRRJ042, and 95BRBA007), the genetic subtypes of which were not previously reported, were subsequently characterized by heteroduplex mobility assay, and the Brazilian subtype variant (B″) was identified based on the restriction fragment length polymorphism on digestion with Fok-I restriction enzyme, as described.[25] Of note, 18 patients were asymptomatic and 6 presented clinical symptoms of AIDS[27] at the time of blood collection for virus isolation. Stock viruses have been kept at −70°C, and expanded only in peripheral blood mononuclear cells (PBMCs) from normal donors, as described.[28] The isolates Ba-L, MN, and T-CSF were a donation of Michael A. Norcross (CBER/FDA, Bethesda, MD), and were grown only in PBMCs from normal donors (Ba-L), or in the T $CD4^+$ tumor cell line PM-1 (MN, T-CSF).

Cells

PBMCs from healthy donors were obtained by density gradient centrifugation (Hystopaque, Sigma Chem. Co., St Louis, MO) from buffy coat preparations and, from these PBMCs, monocyte-derived human macrophages were isolated by plastic adherence. Briefly, 3×10^6 PBMCs were plated in 24-well plates in RPMI without serum for 1 hr, 5% CO_2, 37°C. The nonadherent cells were vigorously washed out, and the adherent cells were maintained with Dulbecco's medium with 10% AB human serum (Sigma), for differentiation in macrophages and for infection. The macrophage purity was above 90%, as checked by FACS analysis using murine anti-CD3-FITC (Pharmingen, San Diego, CA) and anti-CD14-PE (Pharmingen) monoclonal antibodies. The expression of the HIV-1 receptors CD4, CXCR4, and CCR5 on cell membranes of monocytes and macrophages was quantified by immunofluorescence staining. Briefly, cells were detached on Day 0 (monocytes) or after 7 and 10 days of culture (macrophages) by gentle scraping, washed, and incubated for 15 min in phosphate-buffered saline containing 1% bovine serum albumin, 0.1% sodium azide, and 25% human plasma at 4°C. Cells were then incubated with murine anti-CD4-Cy-Chrome (Becton- Dickinson, San Jose, CA), murine anti-CXCR4-PE (R&D Systems, Minneapolis, MN), and murine anti-CCR5-FITC (R&D Systems) monoclonal antibodies for 30 min at 4°C, or with irrelevant isotype controls. Cells were washed and fixed with 1% formaldehyde, and analyzed by flow cytometry (FACSort, Becton-Dickinson).

The T $CD4^+$ tumor cell lines PM-1 (donated by Paolo Lusso, DIBIT, San Rafaelle Scientific Institute, Milan, Italy), MT-2, H9, and SUP-T1 (donated by Michael A. Norcross, CBER/FDA, Bethesda, MD) were cultivated in RPMI 1640 plus 10% fetal bovine serum (FBS) (HyClone, Logan, UT), HEPES, and penicillin/streptomycin, in 25-cm^2 culture flasks, and passaged twice a week. MT-2, H9, and SUP-T1 cells express the molecule CXCR4 on the cell membrane, but are negative for CCR5, while the PM-1 cells are positive for both receptors.[29] Therefore, the first three cells are permissive only to CXCR4-using viruses, while PM-1 cells can be infected by isolates using either coreceptor. The human astroglioma U87 cells stably transfected with CD4 and with CCR5 or with CXCR4 (U87-CD4-CCR5 and U87-CD4-CXCR4, respectively) were donated by Dan Littman (Howard Hughes Medical Institute, New York, NY). They were maintained in Dulbecco's minimal essential medium containing 10% FBS, glutamine, penicillin/streptomycin, puromycin (1 μg/ml, Sigma) and neomycin (G418; 300 μg/ml, Sigma), and were split twice a week, as described.[30]

Macrophage infection

To investigate the ability of the isolates to replicate in monocyte-derived macrophages, these cells were infected with HIV-

1-positive cell-free supernatants after 7 to 10 days of maturation, using 5 to 10 ng/ml of p24 Ag. After overnight incubation, the macrophages were washed, and fresh Dulbecco's medium was added. Viral replication was evaluated by detecting the p24 Ag by ELISA (Dupont, Wilmington, DE) on the culture supernatants each 7 days. Each isolate was tested in cells of two different donors, in duplicate wells. Those still negative, were tested in cells of a third donor. The isolates Ba-L and MN were used as positive and negative controls for macrophage infection, respectively.

MT-2 infection and syncytium formation assays

These two methods were used for detecting the tropism of the isolates for T cell lines. MT-2 cells (5×10^4) were plated in flat-bottom 96-well tissue culture plates, and incubated with HIV-1-positive supernatants (5 to 10 ng/ml of p24 Ag) for 2 to 3 days at 37°C, 5% CO_2, and the infection was monitored by the observation of the cytopathic effect and syncytium formation under an inverted microscope. Viruses were referred to as syncytium-inducing (SI) or non-syncytium-inducing (NSI), according to their ability to infect and form syncytia in MT-2 cells. The isolates Ba-L and MN were used as negative (NSI) and positive (SI) controls for MT-2 infection, respectively. Each isolate was tested in triplicate wells, at least two times. To ensure that the culture supernatants contained viable viruses, PM-1 cells were simultaneously exposed to the same stock viruses, which always resulted in positive infection. The syncytium-inducing ability of the primary isolates was confirmed using the cell fusion assay. For this, PM-1 cells chronically infected with different primary viruses were obtained by mixing PM-1 cells with virus suspensions, as described.[31] Newly infected cells usually formed multinucleated syncytia after 3 to 4 days in culture, developing into chronically infected cell lines by 2 weeks. For cell fusion and syncytium formation, the resultant HIV-1-infected PM-1 cells (10^4) were mixed with uninfected PM-1, MT-2, H9, or SUP-T1 cells (5×10^4) in 96-well plates, and the multinucleated giant cell formation and cytopathic effect were monitored by microscopic observation for 3 to 4 days. PM-1 cells chronically infected with the isolates MN or Ba-L were used as a positive and negative controls for fusion with H9, MT-2, and SUP-T1 cells, respectively. Each isolate was tested in triplicate wells in three different occasions.

Coreceptor usage

The preferential coreceptor usage of the primary viruses was assessed by infecting the set of U87 cells mentioned above. Cells (5×10^4) were seeded in 24-well plates 18 to 24 hr before the assay, and incubated overnight with HIV-1-positive cell-free supernatants. Cells were washed and fresh Dulbecco's medium was added. The chemokine receptor usage was identified by monitoring the cytopathic effect under microscopic observation. When the cytopathic effect was not observed, the absence of virus growth was confirmed by measuring the reverse transcriptase activity in the culture supernatants after 7 to 10 days, as described.[32] Nontransfected U87 cells, as well as cells transfected only with CD4, were also exposed to the same supernatants as negative controls. The CCR5-dependent isolate Ba-L and the CXCR4-using virus MN were used as positive controls for infection of U87-CD4-CCR5 and U87-CD4-CXCR4 cells, respectively. Each isolate was tested in duplicate wells on three different occasions.

Inhibition by dextran sulfate (DS)

The sensitivity of the primary isolates to neutralization by DS was studied by cell fusion assay as described above, but using only uninfected PM-1 as target cells. DS is a negatively charged polysaccharide that inhibits HIV-1 infection through binding to the positively charged V3 loop of cell line-adapted isolates.[33] Chronically infected and uninfected PM-1 cells were mixed in the presence or absence of different concentrations of DS, and the infection was measured by detecting the reverse transcriptase activity on the culture supernatants after 7 to 10 days of culture, as described.[32] For comparison, we used the highly antibody-sensitive laboratory-adapted isolate T-CSF,[17] which was also strongly inhibited by DS in previous experiments (data not shown). Each isolate was tested in triplicate wells on three different occasions.

RESULTS

The biological properties of a panel of 24 Brazilian primary isolates of HIV-1 were studied, using several methodologies, in order to better characterize the HIV-1 strains circulating in different regions of Brazil. The genetic polymorphism and other features of these isolates were previously reported.[24]

Initially, the phenotype of the isolates was determined exposing MT-2 cells to cell-free HIV-1-positive supernatants. We detected that 16 isolates (67%) did not infect or form syncytia (NSI viruses), and 8 (33%) infected and induced syncytia in MT-2 cells (SI isolates) (Table 1).

Continuous cultures of 18 primary isolates were established in PM-1 cells, and their syncytium-inducing properties were confirmed through the ability of infected cells to fuse with uninfected $CD4^+$ T cell lines PM-1, MT-2, H9, and SUP-T1. As expected, all infected PM-1 cells were able to fuse and form syncytia with uninfected PM-1 cells (Table 1). Ten NSI isolates promoted cell fusion only with PM-1 cells, suggesting a CCR5 preferential usage, while the remaining eight isolates induced cell fusion with all types of cell lines, pointing to a CXCR4 tropism for these viruses (Table 1). The isolates 95BRRJ017, 95BRRJ019, 96BRRJ026, 95BRSP004, 95BRSP005, and 95BRSP006 were not tested on cell fusion assay because they induced a massive destruction of PM-1 cells, which prevented the establishment of continuous culture infections and, thus, the cell fusion assays as such.

The cell tropism of the isolates was further evaluated by exposure of monocyte-derived macrophages to HIV-1-positive supernatants. This approach was used because it has been shown that some SI viruses can also replicate in macrophages.[34–36] To verify whether the culture conditions could have influenced the cell expression of HIV-1 receptors and, therefore, the MDM permissiveness to HIV-1 infection, we quantified the molecules CD4, CXCR4, and CCR5 on cell membranes of monocytes and MDM by flow cytometry. We found that the mean fluorescence intensity ($\pm$ standard error of the mean) for cells of three healthy donors just after the cell adherence and after 10 days of culture were, respectively, for CD4: 20 ± 6 and 21 ± 3; for CCR5:

TABLE 1. PHENOTYPE CHARACTERIZATION AND CELL TROPISM OF HIV-1 ISOLATES PREVALENT IN BRAZIL

Isolates	Syncytium formation[a]	Cell fusion[b]				Virus production in macrophages, p24 (pg/ml)[c]	Genotype[d]
		PM-1	MT-2	H9	SUP-T1		
95BRRJ002	NSI	+	−	−	−	73	B
95BRRJ004	NSI	+	−	−	−	710	B
95BRRJ005	NSI	+	−	−	−	330	B
95BRRJ009	NSI	+	−	−	−	107	B″
95BRRJ010	SI	+	+	+	+	Neg.	B″
95BRRJ017	NSI	ND[e]	ND	ND	ND	84	B″
95BRRJ019	NSI	ND	ND	ND	ND	90	B
95BRRJ021	NSI	+	−	−	−	960	F
96BRRJ023	SI	+	+	+	+	Neg.	B″
96BRRJ026	NSI	ND	ND	ND	ND	910	B
97BRRJ040	NSI	+	−	−	−	420	B
97BRRJ042	SI	+	+	+	+	620	B″
95BRSP001	SI	+	+	+	+	470	B″
95BRSP003	SI	+	+	+	+	867	B
95BRSP004	NSI	ND	ND	ND	ND	184	F
95BRSP005	NSI	ND	ND	ND	ND	337	B
95BRSP006	NSI	ND	ND	ND	ND	90	C
95BRSP007	SI	+	+	+	+	520	B
95BRSP008	SI	+	+	+	+	100	B
96BRMG001	NSI	+	−	−	−	196	B
96BRMG002	NSI	+	−	−	−	75	B
96BRMG003	NSI	+	−	−	−	77	B
96BRMG005	NSI	+	−	−	−	60	F
95BRBA007	SI	+	+	+	+	450	B

[a]Syncytium formation in T CD4$^+$ MT-2 cell (SI, syncytium inducing; NSI, non-syncytium inducing).
[b]+, cell fusion and syncytium formation between PM-1-infected cells and uninfected cells; −, no cell fusion observed.
[c]Numbers represent the peak of p24 Ag production; Neg., infection not detected.
[d]Results partially reproduced from Brazilian Network for HIV Isolation and Characterization.[24]
[e]ND, not done.

40 ± 2 and 19 ± 1; for CXCR4: 50 ± 7 and 26 ± 7. Although we detected a decrease in the expression of chemokine receptors in MDM relative to fresh monocytes, the molecules remain expressed at the cell surface. Moreover, we found that the percentage of cells that coexpressed CD4 and its respective chemokine coreceptor, as shown by two-color staining, was even increased after 10 days of differentiation: from 9 ± 1 to 30 ± 5 for CD4/CCR5 and from 15 ± 3 to 19 ± 3 for CD4/CXCR4. Regarding MDM infection, we found that from 24 isolates, 22 (91%) replicated in macrophages (Table 1). Among these viruses, 6 also infected MT-2 cells and promoted cell fusion and syncytium formation with the other tumor cell lines, showing a tropism to primary and tumor-cell lines. Two SI isolates did not grow in macrophages, despite being repeatedly tested in cells from different donors, suggesting a strict T cell line phenotype (Table 1). Of note, macrophage infection with the positive control Ba-L was constantly positive, whereas the negative control MN was invariably negative.

Next, we investigated the preferential coreceptor usage of 18 isolates, exposing U87 cells transfected with CD4 and with chemokine receptors to HIV-1-positive cell-free supernatants. We found (Table 2) that 11 viruses replicated only in CCR5$^+$ cells (R5-tropic viruses), 3 infected only CXCR4$^+$ cells (X4-tropic viruses), whereas 4 used both coreceptors to enter the target cells (R5X4-tropic or dual-tropic viruses). All R5 isolates also replicated in macrophages, but not in MT-2, H9, or SUP-T1 cells. The R5X4 isolates infected both macrophages and tumor cell lines. Regarding the three X4 viruses, they grew in all tumor cell lines used, as expected. Two of them did not infect macrophages, but we observed that the isolate 95BRSP001 was also able to infect these cells, a example of a controversial issue, i.e., the ability of HIV-1 to enter macrophages using the CXCR4 molecule.[34,37] No HIV-1 growth was detected in U87 cells not transfected with chemokine receptors.

In addition, we verified whether the isolates could be blocked by DS, in an attempt to correlate the viruses coreceptor usage with their sensitivity to an agent that mediates HIV-1 inhibitory activity through binding to positively charged V3 loops.[33] We observed (Fig. 1) that two X4 isolates tested were readily neutralized by relatively low concentrations of DS, while the R5 and R5X4 isolates required concentrations of DS four to eight times higher to reach the level of neutralization seen for X4 strains. Among the primary viruses studied, six (95BRRJ009, 95BRRJ010, 95BRRJ017, 96BRRJ023, 95BRRJ042, and 95BRSP001) show the P → W mutation in the tip of the V3 loop. This modification characterizes the Brazilian B subtype, which represents approximately 40% of the HIV-1 isolates circulating in Brazil.[24] We did not find any unique biological property for these isolates, according to the methods used in this work.

TABLE 2. CHEMOKINE RECEPTOR USAGE OF BRAZILIAN ISOLATES OF HIV-1

Isolates	Syncytium formation[a]	Chemokine receptor	
		CCR5[b]	CXCR4
95BRRJ02	NSI	+	−
95BRRJ04	NSI	+	−
95BRRJ05	NSI	+	−
95BRRJ09	NSI	+	−
95BRRJ10	SI	−	+
95BRRJ19	NSI	+	−
95BRRJ21	NSI	+	−
96BRRJ23	SI	−	+
95BRRJ26	NSI	+	−
97BRRJ40	NSI	+	−
97BRRJ42	SI	+	+
95BRSP01	SI	−	+
95BRSP03	SI	+	+
95BRSP07	SI	+	+
96BRMG01	NSI	+	−
96BRMG02	NSI	+	−
96BRMG03	NSI	+	−
95BRBA07	SI	+	+

[a]See footnote a to Table 1.
[b]+, cytopathic effect positive; −, no cytopathic effect and reverse transcriptase activity observed.

DISCUSSION

In this article we describe biological properties of HIV-1 isolates prevalent in Brazil. We studied 24 primary isolates, the genetic polymorphism and preliminary phenotypic evaluation of which were recently reported.[24] Now, we present further data, demonstrating that these isolates are phenotipically similar to those prevalent in other countries, regardless of the genetic diversity. In addition, no distinct characteristics were found for the Brazilian B subtype.

Results of infection of MT-2 cells, showing the predominance of NSI viruses (Table 1), are in agreement with the clinical status of the patients enrolled in the studied cohort, since the great majority of them were asymptomatic at the time of virus isolation, and with less than 2 years of seroconversion. In fact, viruses harbored by asymptomatic HIV-1-positive patients have been described as predominantly NSI.[10,11,38] We extended the observation on the syncytium-inducing properties of our isolates by infecting three other $CD4^+$ tumor cell lines, including the PM-1 cell line, which is permissive to SI or NSI variants.[17,39] The results (Table 1) confirmed the phenotypes NSI or SI found when MT-2 cells were infected by the same viruses and, furthermore, suggest that the HIV-1-mediated syncytium formation is related to the presence of the adequate chemokine receptor on the target cell membrane. In fact, fusion between uninfected PM-1 cells and HIV-1 chronically infected PM-1 cells was invariably positive, regardless of the ability of the virus to use CCR5 or CXCR4. In other words, it seems that all HIV-1 isolates, including the macrophage-tropic and the R5 viruses, can induce syncytium formation in $CD4^+$ tumor cell lines, as long as the target cell expresses the virus preferential coreceptor. The apparent ability of X4- and R5X4-tropic isolates to infect a larger panel of $CD4^+$ tumor cell lines than R5 isolates could be a consequence of a methodological bias, since the receptor CXCR4 is much more frequently expressed than

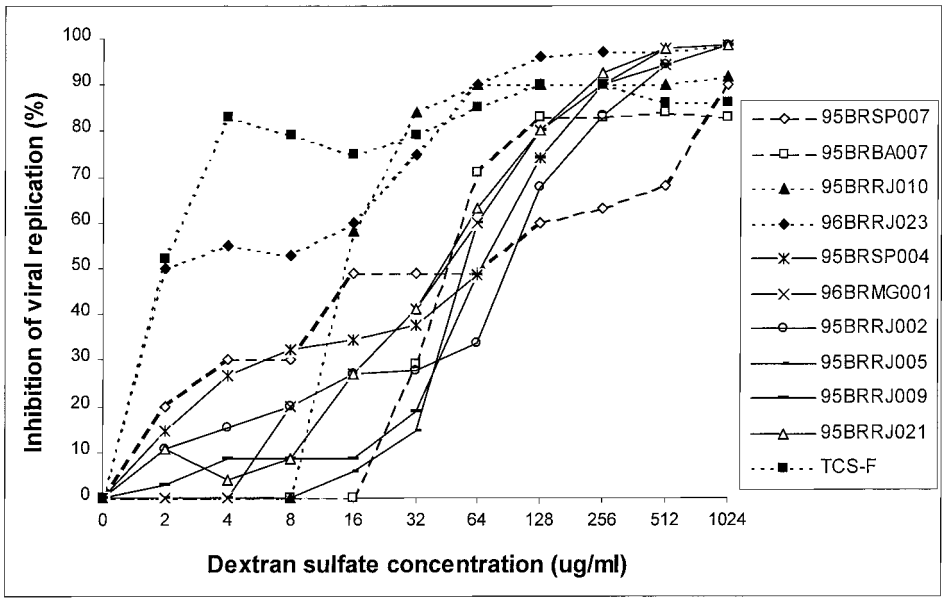

FIG 1. Inhibition of R5-, R5X4-, and X4-tropic primary isolates by DS. Continuously infected PM-1 cells (10^4 per well) were mixed with uninfected PM-1 cells (5×10^4 per well), in the presence or absence of the indicated concentration of DS. Viral replication was measured by detecting the reverse transcriptase activity on the culture supernatants 7 to 10 days later. Data are representative of three similar experiments. Solid lines: R5-tropic isolates; dashed lines: R5X4 isolates; dotted lines: X4-tropic isolates. The X4-tropic isolate T-CSF was used as a control for sensitivity to DS.

CCR5 among the tumor cell lines usually selected for studies of HIV-1 cytopathicity, such as MT-2, H9, and SUP-T1 cells.

Our findings from infection of MDM with HIV-1 highlight the importance of this method for improvement and assurance of the biological characterization of HIV-1 isolates. Using this approach, we could identify six isolates able to grow in MDM and tumor cell lines (Table 1), which otherwise would have been classified just as T cell line tropic isolates if we had limited the phenotypic studies of these isolates to MT-2 infection. Thus, we confirmed that the ability of HIV-1 isolates to infect and form syncytia in $CD4^+$ tumor cell lines does not rule out the ability of these viruses to replicate in macrophages, as reported by other authors.[40–43] In conclusion, it seems more adequate to classify HIV-1 isolates only as NSI or SI viruses when the phenotypic studies are limited to viral infections in MT-2 or other $CD4^+/CCR5^-$ tumor cell lines.

From the three CXCR4-using viruses, only one (95BRSP001) replicated in macrophages. The other two (95BRRJ10 and 95BRRJ23) did not replicate in MDM, even after being tested in cells of three different donors, and using infectious concentrations similar to those that resulted in productive macrophage infections by all CCR5-using isolates and one X4 virus. Moreover, according to our results regarding the expression of CD4 and CXCR4 on MDM cell membrane, the absence of infectivity by these two clinical isolates cannot be explained by lack of expression of one or both HIV-1 receptors. Thus, the inability of these two X4 variants to grow in macrophages may reflect isolate-specific inability to use the molecule CXCR4 for envelope–cell membrane fusion, or to promote the formation of the gp120–CD4–CXCR4 entry complex, as described.[34,37]

The knowledge of chemokine receptor usage is crucial for understanding the cellular tropism of HIV-1 primary isolates. Recently, a new classification of HIV-1 phenotypes was defined, based on the preferential type of coreceptor that the viruses interact with during the infection process.[8] According to this, we found 11 R5-tropic, 3 X4-tropic, and 4 R5X4-tropic (dual tropic) isolates among 18 primary isolates studied. Importantly, the higher frequency of R5 isolates matches the detection of macrophage tropism of the majority of them. Together, the results of MDM infection and chemokine receptor usage reflect the asymptomatic clinical status of the majority of the HIV-1-infected individuals from whom the primary viruses were isolated. As reported by others,[10,15] asymptomatic HIV-1-positive patients harbor predominantly macrophage-tropic and CCR5-using viruses.

Regarding the sensitivity to DS, we found that the two X4-tropic isolates tested were blocked by relatively small concentrations of DS, whereas doses of DS four to eight times higher were required to inhibit R5 or R5X4 variants (Fig. 1). These findings may reflect the predominant presence of basic amino acids in the V3 loop of the X4 viruses, and acidic residues in the V3 region of R5 and R5X4 isolates.[16] Thus, we could envisage that R5 and R5X4 primary viruses will hardly be blocked by negatively charged agents endowed with inhibitory properties based on electrostatic binding to the HIV-1 V3 loop.[44] The relevance of this finding may be controversial, since the clinical isolates could not be considered primary viruses anymore, because they were passed in a tumor cell line (PM-1) for this experiment. Nevertheless, the results seem interesting, as we determined that R5 and R5X4 viruses were clearly much more resistant to DS than the equally passed X4 viruses.

Our findings show that the Brazilian isolates are phenotypically similar to those prevalent in other regions, which could mean that therapeutic strategies based on HIV-1 phenotypic properties, such as inhibitors of CCR5 or CXCR4 entry pathways, would be efficient in Brazil, as in other countries.

ACKNOWLEDGMENTS

This work was supported by the WHO/GPA Global Programme on AIDS (UNAIDS), the Banco do Brasil Foundation, the Brazilian Ministry of Health STD/AIDS Program, and the AIDS Integrated Program, Oswaldo Cruz Foundation, Brazil. The primary virus isolates were obtained from the repository of the Brazilian Network for HIV Isolation and Characterization. The authors are grateful to Hemocentro da Bahia for providing whole blood from normal donors. We thank Elisabeth Deliège-Vasconcelos for secretarial assistance.

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