Neutralization Susceptibility of B Subtype Variant B″ Primary HIV-1 Isolates

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Susceptibility to autologous and heterologous neutralization of primary human immunodeficiency virus (HIV)-1 isolates belonging to subtype B, to the B″-variant of subtype B or to subtype F from infected individuals residing in Rio de Janeiro was assayed. A lower infectivity of the B″- and F isolates when compared to the classical B-subtype HIV-1 isolates was observed. Comparisons of neutralization susceptibilities were carried out for 19 B-subtype, 11 B″-variant and two F-subtype HIV-1 isolates with plasma from autologous and heterologous samples. Frequency of autologous neutralization was slightly lower for B-subtype isolates in comparison to B″-variant isolates. Heterologous intra-subtype neutralization was significantly lower for B-subtype than for the B″-variant or the F-subtype isolates. While B-subtype isolates were neutralized by most anti-F-subtype plasma, F-subtype isolates, although most susceptible to F-subtype antibodies, were highly susceptible to neutralization by anti-B-subtype antibodies. Cross-neutralization for B″-variant and B-subtype isolates was not as extensive as observed for B- and F-subtype isolates. However, the results presented indicate a quite extensive cross-neutralization between Brazilian HIV-1 isolates.

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INTRODUCTION

Sequencing data have demonstrated the presence of human immunodeficiency virus (HIV)-1 clades B, F and C in Brazil, as well as cases of recombinant B/F genomes [1–5], with approximate frequencies of 89% subtype B, 9% subtype F and 2% subtype C of HIV-1 [6]. Sequencing data and analyses with restriction endonuclease enzymes have shown that the Brazilian isolates classified as 'B-subtype' can be divided into three groups: (1) a major group displaying the classical GPGR sequence at the top of the gp120 V3 loop, present in the great majority of the B-subtype isolates from North America and Europe, corresponding to 46.5% of the Brazilian B-subtype isolates; (2) another major group, designated B″-variant of the B-subtype, presenting the sequence GWGR at the top of the gp120 V3 loop, often associated with a Methionine adjacent to this amino acid group to the C-terminal [1–3], present in 39.4% of the Brazilian B-subtype isolates; and (3) isolates with a variable sequence of amino acids at this position (14.1%) [6].

The need for an anti-HIV vaccine in Third World countries is paramount, even after development of triple chemotherapy [7, 8]. Although no absolute correlation has been established as yet, effective anti-HIV-1 vaccines are expected to induce HIV-1-neutralizing antibodies [9, 10] and cytotoxic T-lymphocytic activity [11]. The importance of inducing an immune response capable of controlling replication of the different genotypes of HIV-1 circulating in diverse geographical areas is one of the major problems to be confronted in vaccine development. Although cross-clade neutralization has been described [12–15], a first step towards a reasonable choice of vaccine candidates to be tested is the analysis of the prevalent types of HIV-1 and their susceptibility to the immune response to be induced.

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The present study presents data on the susceptibility of the major genetic subtypes of primary Brazilian HIV-1 isolates against autologous and heterologous neutralizing antibodies.

MATERIALS AND METHODS

Patients. Patients were selected from cohorts of the Evandro Chagas Hospital, IOC, FIOCRUZ, and from the State Employee Hospital, Rio de Janeiro, RJ, Brazil, covering different clinical phases [16], ages, sex and risk groups. Table 1 presents clinical data of the patients involved in this study, from which HIV-1 isolation was achieved.

HIV-1 isolation. We isolated HIV-1 using traditional co-culture of patient peripheral blood mononuclear cells (PBMC) with pre-activated PBMC from normal individuals [17]. Not more than three passages (addition of fresh cells) were carried out for production of viral stocks. Blood donors were tested for HIV infection, human T-cell lymphotropic virus type I (HTLV-I), hepatitis B and C, syphilis and Chagas' Disease.

Neutralization assay. Supernatants collected between the 7th and 21st day of primary co-culture were used as viral stocks. The standard assay employing pre-incubation of viral dilutions with 1:10 and 1:50 diluted plasma, for 1 h at 37°C, followed by addition of phytohaemagglutinin-activated normal human PBMC (10^5 cells per well, mixture of PBMCs from at least two donors), was used [18, 19]. Changes of the medium after 24 and 48 h were carried out in order to eliminate anti-p24 antibodies eventually present in the plasma used [20]. Evaluation of neutralization was carried out by measurement of the HIV-1 p24 antigen (DuPont HIV-1 antigen assay: DuPont de Nemours & Co., Boston, MA, USA) in the culture supernatants on day 7 [18]. Tissue culture infective doses 50% (TCID 50%) were measured simultaneously to determination of neutralization, using the same donor PBMCs. Neutralization results obtained using between 10 and 50 ID_{50} were evaluated. Neutralization was considered positive when a reduction of at least 75% of viral input was detected as measured by p24 concentration [19].

The goat serum anti-SP10 HIV-1 MN V3 loop (catalogue number 1506: AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, Bethesda, MD, USA) was used as neutralization reference control in some of the assays. A pool of plasma from 50 HIV-1 positive individuals, in different phases of disease progression, was also included.

HIV-1 subtype determination. The determination of the genetic subtype of the HIV-1 isolates was carried out using the heteroduplex mobility assay (HMA) with the primers and technique described by Delwart *et al*. [21]. Differentiation between B-subtype and the B″-variant of subtype B was carried out using analysis of Fok 1 restriction enzyme fragments by agarose gel electrophoresis [22].

Statistical analyses. Fisher's exact test including Yates' continuity correction for two-sided *P* values was used for evaluation of neutralization frequencies. The non-parametric unpaired Mann–Whitney *U*-test was used for comparison of the TCID 50% data.

Table 1. Clinical data of HIV-1 isolate donors

	n	HIV-1 B	HIV-1 B″	HIV-1 F
n	32	19	11	2
Sex				
Female	15	8	6	1
Male	17	11	5	1
Age				
Limits (mean)		21–61(36)	22–43(30)	20–38(29)
Transmission				
Heterosexual	16	8	7	1
Bisexual	5	4		1
Homosexual	9	6	3	
Transfusion	1		1	
Not known	1	1		
Clinical classification[a]				
II	20	10	10	
III	3	1	1	1
IV	9	8		1
No. CD4/mm^3				
>400	14	5	8	1
200–400	5	4	1	
<200	9	6	2	1
Not known	4	4[b]		

[a] See Ref. 16.

[b] Three individuals classified as asymptomatic and one as AIDS.

RESULTS

HIV-1 isolation

A total of 61 HIV-1 infected individuals donated blood for this study. Typing by HMA carried out on PBMCs indicated 33 individuals infected with HIV-1 subtype B, 21 with variant B″ and seven with subtype F. Although some differences of clinical data between the groups existed in addition to the numerical differences, such as a higher percentage of female individuals in the group infected with B″ HIV-1 (67%, versus 54% in B and 57% in F) with a correspondingly lower percentage of HIV-1 transmission via homosexual or bisexual contacts (19% in group B″, 39% in group B and 43% in group F) and a higher percentage of individuals in a more advanced stage of disease in the subtype B HIV-1 group, these differences were not of statistical significance.

Isolation of HIV-1 was achieved for 48 samples, with a percentage of isolation of 71% of the B-subtype HIV-1, 63% of the B″-variant HIV-1 and 37% of the F-subtype HIV-1. However, determinations of the infectivity (TCID 50%) showed that for 31% of the B-subtype, 31% of variant B″ and 50% of the F-subtype isolates the infectivity was too low to allow neutralization experiments to be properly evaluated.

The mean TCID 50% of the 18 B-subtype isolates was higher than the mean TCID 50% value for the 11 B″-variant isolates or the two F-subtype isolates. Statistical analysis indicated that the TCID 50% of the group of B″-variant isolates was significantly lower than that determined for the group of B-subtype HIV-1 isolates ($P = 0.0026$). However, when comparing isolates from individuals with similar clinical characteristics, this difference was shown to be due mostly to the greater number of symptomatic individuals in the group from which the classical HIV-1 B-isolates were derived (Fig. 1).

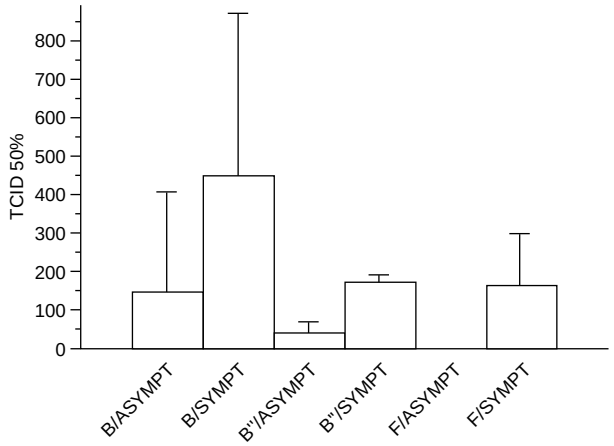

Fig. 1. Infectivity (TCID 50%) of primary HIV-1 isolates typed as clade B ($n = 29$) isolate from asymptomatic ('asympt'; $n = 19$) or symptomatic ('sympt'; $n = 10$) individuals, isolates typed as B″ variant of subtype B ($n = 15$ and 3, respectively) or subtype F ($n = 3$ and 4, respectively).

Susceptibility of primary HIV-1 to neutralization

To allow a comparison of the susceptibility to neutralization of primary HIV-1 isolates of the different subtypes, a summary of results obtained analyzing the neutralization of primary isolates is presented in Table 2, with an overview of the data available. Results were obtained using different individual plasma/virus combinations. Results are shown as >50%, >75% and >90% neutralization of viral input.

Susceptibility to autologous neutralization

As shown in Table 2, approximately half of the isolates were susceptible to autologous 75% neutralization, similar to data reported for other primary HIV-1 isolates [18, 23–25], although the autologous neutralization of the B″-variant isolates was slightly higher than that observed for the B-subtype isolates. The low number of F-subtype isolates available for neutralization analyses does not allow an effective comparison with the other two HIV-1 subtypes studied.

Susceptibility to heterologous neutralization

Heterologous intra-subtype neutralization was higher for the F-subtype HIV-1 isolates, in comparison to neutralization by the anti-B or anti-B″ antibodies (no statistical significance at 90% neutralization). The B″-variant HIV-1 isolates were neutralized to similar extent both by anti-B-subtype and anti-B″-variant antibodies. However, subtype B isolates were less susceptible to neutralization by the anti-B-subtype antibodies than by anti-B″-variant antibodies ($P = 0.0279$, 90% neutralization) or by anti-F-subtype antibodies ($P = 0.0143$, 90% neutralization). A chequerboard neutralization, shown in Table 3, indicates that no absolute neutralization types could be established. Comparing efficiencies of intra-subtype neutralization, the B-subtype neutralization by anti-B-subtype antibodies was significantly less frequent than neutralization of B″-variant isolates by anti-B″-variant antibodies ($P = 0.0012$, 90% neutralization) or than neutralization of the two F-subtype isolates by anti-F-subtype antibodies ($P = 0.0010$, 90% neutralization).

Table 2. Summary of the neutralization data obtained. Data are presented in terms of number of HIV-1 isolates neutralized to >50%, >75% and >90% by autologous, heterologous or reference plasma, out of the total number of assays carried out

	Neut	Aut Neut	Neut by het anti-B	Neut by het anti-B″	Neut by het anti-F	Neut by NIH#1506	Neut by pool
HIV-1	50%	9/19	20/31	14/21	10/11	2/8	19/27
clade B	75%	8/19	12/31	11/21	8/11	2/8	17/27
	90%	4/19	5/31	9/21	6/11	2/8	15/27
HIV-1	50%	7/11	12/15	18/23	6/9	5/5	16/19
clade B″	75%	7/11	10/15	17/23	6/9	3/5	14/19
	90%	6/11	10/15	14/23	2/9	1/5	11/19
HIV-1	50%	1/2	8/10	4/8	7/7	2/2	5/5
clade F	75%	1/2	7/10	3/8	7/7	2/2	5/5
	90%	1/2	7/10	3/8	6/7	1/2	4/5
HIV-1	50%		20/27	21/22	6/6	+	+
strain MN	75%		18/27	20/22	6/6	+	+
clade B	90%		16/27	16/22	6/6	+	+

Neut, neutralization; Aut Neut, autologous neutralization; Neut by het anti-B anti-B″ anti-F, neutralization by heterologous plasma from individuals infected with the corresponding HIV-1 subtypes; NIH#1506, goat anti-V3 (HIV-1 MN) serum; Pool, pool of 50 plasma from HIV-1 infected individuals residing in Rio de Janeiro.

Comparison to HIV-1 MN neutralization

Neutralization frequency of the MN strain was higher than that observed for the primary B-subtype isolates by anti-B-subtype antibodies ($P = 0.0009$ at 90% neutralization), or than B″-isolates by anti-B″ or than F isolates by anti-F antibodies (no statistical significance). Comparison of HIV-1 MN neutralization efficacy by the different plasma indicated a more frequent neutralization of the HIV-1 MN strain by plasma from individuals infected with B″-variant or F-subtype HIV-1 than by plasma from individuals infected with B-subtype HIV-1 (no statistical significance).

Neutralization potency

Comparing the effectiveness of the plasma from individuals infected with different HIV-1 subtypes, no clear-cut conclusions as to 'most potent' or 'least potent' antibodies can be drawn. Thus, although both the B″-variant ($P = 0.0017$, 90% neutralization) and the F-subtype isolates ($P = 0.0028$) were more effectively neutralized by the anti-B-subtype antibodies than were the B-subtype isolates, the anti-F-subtype antibodies were very potent against both B- and F-subtype isolates, while the anti-B″-variant antibodies were more efficient in neutralizing the heterologous B″-isolates than isolates of the B- or F-subtypes.

Neutralization by control antibodies

The reference antiserum NIH # 1506, a goat anti-V3 (HIV-1 MN) antiserum, was fairly inefficient in neutralizing the primary HIV-1 isolates (20–50% primary isolates at 90% neutralization), even at the high concentration used (1:100 dilution), 10 times higher than that needed to inhibit the reference HIV-1 MN strain. No correlation between susceptibility to neutralization by this reference antiserum and genetic subtype of HIV-1 isolates was observed. Neutralization of the primary isolates by a control pool of Brazilian HIV plasma was equally high for the different groups of HIV-1 subtype isolates.

Table 3. Chequerboard neutralization of primary B and B′ HIV-1 isolates by B and B″ plasma. Percentage neutralization obtained at plasma dilutions of 1:10 and (1:50) are indicated. Clinical classification of plasma donors is indicated

			HIV-1 # 2	HIV-1 # 10	MN stock
	TCID 50%		150	125	128000
	IU 50		15	12.5	12.8
Plasma	Clin*		B	B″	B
#1	II	B	11	69	75 (92)
#2	II	B	94 (93)	0	92 (95)
#3	II	B	41 (0)	55	70 (38)
#4	II	B	99 (98)	93 (97)	98 (65)
#5	IV	B	75 (97)	84	13 (25)
#6	II	B″	100 (23)	77 (100)	97 (55)
#7	II	B″	60	67	100
#8	II	B″	97 (94)	66	25
#9	II	B″	100 (100)	92 (95)	100
#10	IV	B″	100 (100)	77 (97)	94 (91)

* Clin, clinical classification [16]; TCID 50%, tissue culture infective dose 50% in this assay; IU, infectious units employed.

Chequerboard neutralization

An example of a chequerboard neutralization comparing neutralization of representative HIV-1 isolates from the two more prevalent B-subtype HIV-1 isolates (B and B″) and of the reference HIV-1 strain MN by plasma derived from individuals infected with different HIV-1 subtypes/variants is shown in Table 3. As can be seen even in this limited example, no clear-cut characterization of subtype neutralization susceptibility or neutralization serotype can discerned.

DISCUSSION

As expected from prevalence data [6], HIV-1 isolation of B-subtype and B″-variant HIV-1 isolates was achieved more frequently than the F-subtype HIV-1. Accordingly, the three groups of HIV-1 isolates were of different sizes, but clinical characteristics did not differ significantly between groups. The slightly higher frequency of individuals in a more advanced stage of disease in the B-subtype group may account for the greater number of highly infective variants in this group, allowing the testing of neutralization susceptibilities of more B-subtype isolates. Indeed, the mean TCID 50% of the group of B″- and F-isolates was significantly lower than that determined for the group of B-subtype HIV-1 isolates ($P = 0.0026$). However, although infectivity of B″ HIV-1 isolates was lower than observed for the mean of the B HIV-1 isolates, the infectivity of the B isolates to pre-activated PBMCs was extremely variable, with standard deviation as high as mean infectivity values (Fig. 1).

The significance of neutralization percentages below 75% has been shown to be doubtful in our hands: in 52 assays carried out on different dates (using different host cell donors) with two identical samples of frozen (−70°C) viral stocks, an 11% error was observed only when results below 75% neutralization were compared. These results lead us to determine 75% neutralization as a reproducible positive neutralization.

Comparative analyses of neutralization susceptibilities of primary HIV-1 isolates and of potencies of plasma from individuals infected with different genetic HIV-1 subtypes have indicated a lack of correlation to genetic subtype-specificity [12, 13, 26, 27], with frequent cross-neutralization [28–30]. Results obtained in this study showed no difference in overall susceptibility to neutralization: while a significantly lower susceptibility of B-subtype HIV-1 was observed towards heterologous intra-subtype neutralization in comparison to plasma

from individuals infected with B″- or F-isolates, the B-subtype isolates were more susceptible to neutralization by plasma from individuals infected with F-subtype HIV-1 than were B″-variant primary isolates. Similarly, although F-subtype isolates were highly susceptible to neutralization by anti-B- and anti-F-subtype antibodies, neutralization by anti-B″-variant antibodies was much lower. Furthermore, although a fairly low frequency of neutralization of B-subtype isolates by anti-B-subtype antibodies occurred, these anti-B-subtype antibodies were quite potent in neutralizing B″- or F-isolates. These results appear to confirm the specific escape from neutralization described previously [15, 18], as more of the B HIV-1 isolates were obtained from patients in more advanced stages of disease progression than B″ or F primary HIV-1 isolates. However, this escape appears to be highly specific, and the heterologous neutralization susceptibility results presented here raise hopes for effectivity of passively administered cocktails of neutralizing antibodies, at least for specific therapy such as during pregnancy or parturition.

The lack of correlation between neutralization specificity and HIV-1 genotype has not been so extreme when antibody binding studies are correlated to genotype analyses [31]. The Brazilian B″ variant can be fairly well distinguished from the classical B-subtype by binding to the corresponding synthetic B″- or B-V3 peptides [32, 33]. The neutralization susceptibility of B″-variant HIV-1 isolates towards B- or F-subtype plasma indicates that epitopes different from the V3 must be important for inducing antibodies capable of cross-neutralization.

The HIV-1 strain MN, adapted to growth in T-cell lines, was more susceptible to neutralization than were the primary Brazilian HIV-1 isolates tested. Although the HIV-1 MN strain belongs to the B-subtype, it was less susceptible to anti-B-subtype antibodies than to plasma from Brazilian individuals infected with B″ or F HIV-1. The higher potency of the anti-V3-serum NIH no. 1506 against B″-and F-isolates was statistically significant only at 50% neutralization ($P = 0.0210$), but the similarly low neutralization frequency at 90% neutralization indicates an overall low susceptibility of Brazilian primary HIV-1 isolates to neutralization by this reagent.

The extensive cross-neutralization observed between B-, B″- and F-isolates is fortunate, as it permits hope that vaccine candidates designed using the classical B-subtype HIV-1 will be able to protect against infection by the more frequent subtypes circulating in Brazil.

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