Women Infected with HIV Type 1 Brazilian Variant, Subtype B (B′-GWGR Motif) Have Slower Progression to AIDS, Compared with Patients Infected with Subtype B (B-GPGR Motif)

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Introduction. The Brazilian variant of human immunodeficiency virus (HIV) type 1 (HIV-1) subtype B (serotype B′-GWGR) has a tryptophan replacing a proline in position 328 of the HIV-1 envelope, a feature that may induce a different HIV disease progression. We aimed to evaluate the role of the B subtypes of HIV-1 (serotypes B-GPGR and B′-GWGR) on HIV disease progression.

Methods. A total of 137 HIV-infected individuals who had been admitted to the hospital were tested with an anti-V3 serologic assay, using peptides representing 2 HIV-1 subtype B strains, MN and SF2, and 2 Brazilian variant B′-GWGR strains, BR1 and BR2.

Results. Of 137 serum samples tested with the anti-V3 serologic assay, 4 (3%) yielded indeterminate results, 74 (54%; from 25 women and 49 men) were found to be B-GPGR, and 59 (43%; from 20 women and 39 men) were found to be the B′-GWGR variant. In general, a longer interval from the first known positive HIV test result to an AIDS-defining event was observed in the B′-GWGR group than in the B-GPGR group (21 vs. 7 months). The $CD4^+$ T cell counts were higher in the B′-GWGR group (median $CD4^+$ T cell count, 65 vs. 31 cells/mm^3; $P = .01$), and women infected with the B′-GWGR variant were less likely to die than were men infected with the same variant ($P = .01$). The median viral load in the B′-GWGR group was 3.395 copies/mL, compared with 39.350 copies/mL in the B-GPGR group ($P = .01$).

Conclusions. Taken together, our results indicate that B′-GWGR–infected women may have more-favorable outcomes than B-GPGR–infected subjects.

HIV-1 is a complex retrovirus characterized by extensive genetic variability [1, 2]. In the structural *env* gene, 5 conserved regions (C1–C5) and 5 hypervariable regions (V1–V5) have been identified [3, 4]. The biological relevance of genetic variations in the *env* gene is associated with the central role of the envelope glycoprotein gp120 in the virus-host interaction, particularly with regard to the binding of the virus to the CD4 molecule expressed on the cell surface of human T lymphocytes [5–8, 11]. After initial binding of gp120 to the CD4 molecule, conformational changes in the envelope and engagement of chemokine receptors, usually CCR5 or CXCR4, are required for viral entry [9]. The third hypervariable region (V3) of gp120 typically consists of 35 amino acids (range, 31–39), and plays a number of important biological roles [9, 10]. V3 is a major antigenic epitope loop that is essential for virus infectivity [5–8], is the main neutralizing determinant, and is a target for type-specific anti–HIV-1 neutralizing antibodies [7, 11].

On the basis of phylogenetic analyses of gp120, nine different circulating HIV-1 group M genetic subtypes have been recognized (A–D, F–H, J, and K) [12, 13]. In Brazil, 4 subtypes (B, C, D, and F) [14] and the recombinant B/F and B/C forms, all of which belong to group M, have been described [12, 13, 15]. Molecular

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studies have shown that 2 genetically and antigenically distinct strains of HIV-1 subtype B cocirculate in Brazil [15, 16]. One strain of subtype B has a GPGR motif in the crown of the V3 loop region similar to that in HIV-1 isolates from the United States and Europe [15–19]. The Brazilian variant, named serotype B′-GWGR, has a unique signature in the tip of the V3 loop [16, 17], with a tryptophan replacing a proline at the position 328 on the HIV-1 envelope [20]. Molecular typing and V3 serologic testing have revealed that this variant accounts for almost one-half of HIV-1 subtype B infections in Brazil [17–21].

The role of genetic subtypes in disease progression, vaccine development, and transmission is of great interest [22]. Serologic assays that measure functional binding antibody to whole viruses or epitopes have been used widely in epidemiological studies of numerous viral infections [23–25]. Growing evidence indicates that genetic variability of HIV is associated with differences in the rate of transmission and disease progression that might influence the dynamic of the HIV epidemic [26, 27]. In this study, we analyzed the association of the variants of HIV-1 subtype B, on the basis of the V3 motif [28, 29], with clinical status [30], mode of transmission, patient sex, disease progression, and clinical outcome.

MATERIAL AND METHODS

Serum samples were obtained from all 137 HIV-1–infected patients admitted to Emilio Ribas Institute (São Paulo, Brazil) during the period from 1 October through 30 October 2002. The reasons for admission were death risk, diagnostic investigation, or need for intravenous medication. Demographic and clinical data for 130 of these patients were extracted from clinical records or were obtained through direct interview. The ethics committee of Emilio Ribas Institute approved the study protocol.

We used a previously described V3 serologic assay to identify the Brazilian variant and to assess the avidity of V3 antibodies [17, 28]. In brief, biotinylated peptides (kindly provided by Hugo Guevara, California Department of Public Health, Richmond) based on the V3 loop consensus sequence from subtype B (consensus B, NTRKSIHIGPGRAFY) and 1 synthetic peptide based on the consensus sequence of the Brazilian variant subtype B strain (strain BR1, NTRKSIHIG**W**GRA) were captured onto avidin-coated (Vector Labs) 96-well plates (Corning Costar). Serial 4-fold dilutions of test sera were added to duplicate plates, one of which was washed 5 times with a hyperosmolar (8 M) urea solution–0.05% tween 20 (Sigma), and the other of which was washed 5 times in PBS1X-0.05% tween 20. Peroxidase-labeled anti-human IgG (Kikeguard and Perryga) was added, and the plates were incubated for 1 h. The reaction was developed with a hydrogen peroxide substrate and the tetramethyl benzidine chromogen (3, 3′, 5, 5′–tetramethyl benzidine; Sigma). The plates were read at 450 nm, and the end point titers were interpolated from the linear portion of the titration curve, yielding a mean absorbance of 0.5 optical density units. The high-avidity anti-V3 antibody index was calculated using the dilution end point in the 8 M urea-washed plate divided by the dilution end point in the PBS-washed plate. Antibodies with a high-avidity anti-V3 antibody index >50% were considered to possess higher affinity. $CD4^+$ T cell counts were determined by flow cytometry with commercial monoclonal antibodies (Beckman Coulter), and RNA plasma viral loads were determined using NASBA kits (Organon Teknika).

AIDS-defining events were determined in accordance with guidelines of the Centers for Disease Control and Prevention [20]. Differences between patients in the B′-GWGR and B-GPGR groups with regard to characteristics and laboratory values were tested for statistical significance using Yates' corrected χ^2 test for proportions or the nonparametric Kruskal-Wallis test for continuous variables. The time of known HIV infection was considered to be the time that had elapsed between the first documented positive result of an HIV serologic test and admission to the hospital, which, in the current study, always corresponded to October 2002. The time to AIDS diagnosis was defined as the time that had elapsed between the first positive serologic test result and the occurrence of the first AIDS-defining event. ORs and 95% CIs were calculated with the aid of the free EpiInfo software, version 6.04c (Centers for Disease Control and Prevention).

RESULTS

Seventy-four serum samples (54%) were typed as B-GPGR, 59 (43%) were typed as B′-GWGR, and 4 (3%) yielded indeterminate results. Demographic and clinical data are summarized in table 1. There were no differences between groups with regard to the patients' sex, age, time of HIV diagnosis, or time of hospitalization. The median time from diagnosis of HIV infection and occurrence of an AIDS-defining event was 21 months for patients in the B′-GWGR group, compared with 7 months for those in the B-GPGR group ($P = .001$). Women infected with the B′-GWGR variant were more likely to survive than were women infected with the B-GPGR variant ($P = .005$). None of the women infected with the B′-GWGR variant died, whereas 14 men infected with the same serotype died ($P = .01$). $CD4^+$ T cell counts were measured for 124 patients during the hospitalization period; the median $CD4^+$ T cell counts were 65 and 31 cells/mm^3 for the B′-GWGR and B-GPGR groups, respectively ($P = .01$). Viral load data were available for 130 patients; in the B′-GWGR group, the median viral load was 3395 copies/mL, compared with 39,350 copies/mL in the B-GPGR group ($P = .02$).

The mean anti-V3 titer was higher in the B′-GWGR group than in the B-GPGR group, and women in the B′-GWGR group

Table 1. Baseline covariant distribution, according to viral serotype, in HIV-infected patients from São Paulo, Brazil, during October 2002.

Characteristic	HIV subtype B		OR (95% CI)	*P*
	B′-GWGR group (*n* = 59)	B-GPGR group (*n* = 74)		
No. of female/male subjects	20/39	25/49	1.01 (0.46–2.20)	.8
Age, mean years ± SD	37 ± 9	37 ± 8	…	NS
Duration of known HIV infection, mean months ± SD[a]	66 ± 62	49 ± 56	…	NS
Time to AIDS progression, mean months ± SD[b]	21 ± 41	7 ± 29	…	.001
Time from hospitalization to death, mean days ± SD	29 ± 31	56 ± 45	…	.05
No. of deaths				
Women	0	8	0.00 (0.00–0.84)	.01
Men	14	14	…	
Absolute $CD4^+$ T cell count, median cells/mm^3[c]			…	.01
All subjects	65	31	…	
Women	94	26	…	.04
Men	65	33	…	
HIV RNA level, median $\log_{10}$ copies/mL[d]	3395	39,350	…	.02
Median anti-V3 titer[e]				
All subjects	2527	2718	…	NS
Women	2344	1584	…	
Men	3683	3511	…	
Median HAAV3 index, %	25	28	…	NS

NOTE. *P* values were determined by nonparametric Mann-Whitney *U* test. HAAV3, high-avidity anti-V3 antibody; NS, not statistically significant.

[a] Defined as the time from the first known positive HIV test result to admission to the hospital.

[b] Defined as the time from the first positive HIV test result to the first opportunistic infection and/or neoplastic AIDS-defining event.

[c] Data were available for 57 patients in the B′-GWGR group and 67 patients in the B-GPGR group.

[d] Data were available for 56 patients in the B′-GWGR group and 74 patients in the B-GPGR group.

[e] Median values were obtained using BR1 peptide for patients in the B′-GWGR group and the MN peptide (with a PBS wash) for those in the B-GPGR group.

presented with titers 3 times higher, compared with women in the B-GPGR group, although that difference did not reach statistical significance.

Table 2 shows epidemiological and clinical features of patients. The 2 patient groups had similar rates of adherence to antiretroviral therapy, as well as similar proportions of patients who took antiretroviral therapy before hospitalization (if it had been used prior to hospitalization), who received *Pneumocystis jiroveci* pneumonia prophylaxis with sulfa, and who received antiretroviral therapy during hospitalization. For 25 patients, information on these characteristics was not available.

DISCUSSION

Some studies have questioned the ability of the subtypes of HIV-1 or their variants to alter the natural history of HIV infection/AIDS [23]. In spite of the inherent difficulties involved in studying this, clarification of the role of subtypes is helpful for understanding the HIV epidemic in our country and, perhaps, for the development of anti-HIV vaccines. Low-cost techniques and the use of plasma and/or serum samples may prove to be useful for the evaluation of large numbers of patients.

In the current study, there were no significant differences between the B′-GWGR and B-GPGR groups in terms of sex, age, time from the first positive HIV serologic test result to hospital admission, or previous use of HAART. However, a significantly different interval from the first known HIV-positive test result to the onset of AIDS was observed among the 2 groups of patients. Patients who were infected with the B′-GWGR variant developed AIDS 3 times slower than patients infected with the B-GPGR variant. These findings corroborate previous studies from a Rio de Janeiro group and from our own group, suggesting a better prognosis for patients who are infected with the B′-GWGR variant [18, 27]. Santoro-Lopes et al. [27] reported that 32% of the patients in their study who were infected with the B-GPGR variant had progression to AIDS, compared with 22% of the patients who were infected with the B′-GWGR variant. Similarly, in a cohort study from São Paulo, we found a 2-fold lower risk of AIDS development among asymptomatic, untreated patients who were infected

Table 2. Univariate analysis of some epidemiological and clinical parameters among B′-GWGR– and B-GPGR–infected patients.

Variable	B′-GWGR group (n = 59)	B-GPGR group (n = 74)	P
Risk group, no. (%) of patients			
Heterosexual	25 (42)	29 (40)	NS
Injection drug user	17 (29)	12 (15)	
Men who have sex with men	7 (12)	10 (14)	
Unknown or other	10 (17)	23 (31)	
Opportunistic infection during hospitalization			
Pulmonary tuberculosis	15	18	NS
Toxoplasmosis encephalitis	12	19	
Pneumocystis jiroveci infection	11	15	
Other[a]	18	26	
Antiretroviral therapy			
Use	38	51	NS
No use	14	15	
Irregular use	3	6	
No data	4	3	
HCV coinfection	10	8	NS
P. jiroveci pneumonia prophylaxis			
Use	21	27	NS
No use	31	44	

NOTE. Data are no. of patients, unless otherwise indicated. HCV, hepatitis C virus; NS, not statistically significant.

[a] Bronchopneumonitis, neurocryptococcosis, neurotuberculosis, and Kaposi sarcoma.

with the B′-GWGR variant, compared with patients who were infected with the B-GPGR variant [3]. More recently, in another cohort study, B′-GWGR–infected patients had significantly higher avidity to anti-V3 antibodies, higher CD4 T cell counts, and lower RNA viral loads than did B-GPGR–infected subjects [24].

The studies cited above did not evaluate the possible existence of associations between serotypes and the patient's sex. Our findings point to the existence of a difference between sexes with regard to the number of patients who were infected with the B′-GWGR variant. Of the 36 patients who died, 14 were infected with the B′-GWGR variant, and none were female ($P = .01$). In addition, women infected with the B′-GWGR variant had a much lower risk of hospitalization, compared with women infected with the B-GPGR variant ($P = .006$).

The analysis of some laboratory values, such as $CD4^+$ T cell counts and HIV RNA viral loads, revealed that both men and women infected with the B′-GWGR variant presented with higher $CD4^+$ T cell counts at hospitalization, compared with those who were infected with the B-GPGR variant ($P = .01$). The viral load was lower, the V3 antibody levels were higher, and there was a trend towards longer asymptomatic periods after infection among those infected with the B′-GWGR subtype, compared with B-GPGR–infected persons. These findings support the notion that the B′-GWGR variant is less pathogenic than the B-GPGR variant, and our results are in accordance with the results obtained by another group [27].

There is a general view that, after the commencement of antiretroviral therapy, HIV infection/AIDS is a manageable disease and that death became a rarer outcome in developed countries and in countries, such as Brazil, where antiretroviral therapy is freely available. However, diagnosis of AIDS <12 weeks before hospitalization is associated with death [31, 32]. In the present study, we aimed to determine whether the hospital rate of mortality for AIDS in Brazil was influenced by the infecting variant of serotype B of HIV-1.

Some hypotheses can be advanced to explain the slower progression to AIDS among B′-GWGR–infected patients, particularly women. In this study, the mean anti-V3 antibody levels among women infected with the B′-GWGR variant were 2–3-times higher than the levels among men and women infected with the B-GPGR variant (data not shown). These antibodies may make a more efficient attachment to the V3 loop, preventing the HIV from binding to the coreceptors on the target cell over time. These evolving responses may contribute to prolonged survival during HIV-1 disease progression among B′-GWGR–infected patients. It is conceivable that some female hormones, such as estrogens, may provide protection for women with a B′-GWGR motif. In fact, women produce more-vigorous cellular and humoral immune reactions and are more

resistant to certain infections [33]. More recently, it has been shown that women in Tanzania infected with subtype D have a higher risk of disease progression than do women infected with subtypes A or C [34]. Although the effects of female hormones on disease progression have not been well established [35], it is conceivable that they can exert an influence on the progression of disease produced by variant strains, such as B′-GWGR; this requires further investigation.

Our findings are intriguing but should be viewed with caution, because potential confounders, such as the time of acquisition of HIV infection, were not addressed. Further studies involving coreceptor use for viral entry in the 2 strains should be performed, because the V3 tip region is used for coreceptor binding in the target cell.

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