Original Report

HIV disease progression: is the Brazilian variant subtype B' (GWGR motif) less pathogenic than US/European subtype B (GPGR)?

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Background: The aim of this study was to investigate differences in HIV disease progression in patients infected with HIV subtype B with a GPGR motif in the V3 loop region (B-GPGR) versus the Brazilian subtype B variant with a GWGR motif (B'-GWGR).

Materials and Methods: Patients were enrolled in an ongoing cohort study at the University of São Paulo Dermatology Clinic in Sao Paulo, Brazil. V3 serology was performed by enzyme immunoassay with peptides representing two HIV subtype B strains, MN and SF2, and two Brazilian variant B'-GWGR strains. The incidence of AIDS-defining events was calculated, and Cox proportional hazards regression was used to estimate adjusted risk ratios.

Results: Of the samples from 114 patients studied, 23 (20%) were classified as B'-GWGR motif, and 91 (80%) as B-GPGR motif. Patients with T CD4$^+$ cell counts less than 200 cells/mm^3 or 200–400 cells/mm^3 experienced an increased incidence of AIDS-defining events compared with patients who entered the cohort with T CD4$^+$ cell counts greater than 400 cells/mm^3. In a proportional hazard model including age, gender, T CD4$^+$ cell count at entry into the cohort, and V3 serology, GWGR reactivity was associated with a decreased hazard rate for presenting an AIDS-defining condition during follow-up. Three patients in the group with GPGR serology died after experiencing an AIDS-defining event. None of the patients with GWGR serology died during follow-up.

Discussion: Survival analysis showed that patients infected with the Brazilian subtype B variant with a GWGR motif in the V3 loop had lower risk, adjusted for initial CD4$^+$ cell count, of AIDS-defining events than patients infected with subtype B-GPGR strains.

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INTRODUCTION

Viral characteristics have been shown to influence the pathogenesis of human immunodeficiency virus (HIV) infection or progression to acquired immune deficiency syndrome (AIDS). For example, genetic alterations, such as *nef* gene deletion, were shown to be associated with a lower rate of depletion of T CD4$^+$ cells during follow-up,[1] and HIV-2 seems to be less pathogenic than HIV-1.[2] These findings may be related to the capacity of the virus to induce syncitia, differences in coreceptor usage, or other mechanisms.[3]

HIV-1 genetic variation has been assessed using molecular biology, mainly through PCR/heteroduplex gel mobility protocols. These approaches, in addition to DNA sequencing data, have demonstrated that HIV-1 may be classified into at least 10 different genotypes, designated A–J, based on the sequence of the envelope gene.[4] In Brazil, the majority of HIV-1 infections are subtype B, followed by F and the newly emerging subtypes C and D in southern and southeastern regions of the country.[5,6] The city of São Paulo has the highest number of AIDS cases of any city in Brazil, and the state of São Paulo accounts for as much as 40% of all AIDS cases reported in Brazil during the last 20 years.[7] There are at least three different genetic HIV-1 subtypes (B, F and C) co-circulating in the city and state of São Paulo, with subtype B accounting for more than 90% of infections.[5,8]

Complementary to genotype classification, antibodies against the V3 loop region, an important target for humoral and cellular immune responses, have been

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used to serologically discriminate antigenic subtypes.[4] Two antigenically distinct variants of HIV-1 subtype B have been described, mainly in Thailand and Brazil.[9,10] Although the V3 loop region is highly divergent in Brazil, especially in the 15 amino acids of the tip, two genetically and antigenically distinct strains of subtype B account for the majority of HIV-1 subtype B infections: one strain has the GPGR motif in the crown of the V3 loop region, similar to US/European isolates (B-GPGR), whereas the other has a GWGR motif that is unique to Brazil and Latin America (B'-GWGR).[10–13] It has been shown that the Brazilian variant, B'-GWGR, is widely disseminated and represents almost half of the HIV-1-infected subtype B population in some areas.[14,15] A previous report suggested that the rate of progression to AIDS was lower among B'-GWGR-infected than among B-GPGR-infected patients before and after the initiation of antiretroviral therapy (ART).[16] Using a different cohort of HIV-1-infected Brazilian patients, the effect of differences between the two strain types on the rate of AIDS progression can be examined during the natural history of HIV, prior to initiation of highly active antiretroviral therapy (HAART).

We previously described a modified enzyme immunoassay (EIA) based on V3 reactivity to discriminate between subtype B GPGR and B-variant B'-GWGR.[14,15] The objective of the current study was to compare disease outcomes prior to the initiation of ART in patients infected with the B-GPGR strain or B'-GWGR strain.

MATERIALS AND METHODS

The study was conducted at the ambulatory service of the secondary immunodeficiency clinic of Hospital das Clinicas, the largest teaching and research hospital in Latin America. In total, 219 HIV-1-infected subjects were enrolled in a cohort to study the natural history of HIV infection in São Paulo.[17] The first cases were recruited in October 1987 and the last in December 2000. Demographic and clinical data were obtained from clinical charts or direct interview. The Ethical Committee of Hospital das Clínicas, University of Sao Paulo (HCFMUSP), approved the study protocol.

V3 serology was done in accordance with previously published assays.[14,15] Briefly, biotinylated peptides based on the V3 loop consensus sequences from two subtype B strains, MN and SF2, and two synthetic peptides based on the consensus sequence of the Brazilian variant subtype B strain were captured in avidin-coated 96-well plates. Serial four-fold dilutions of test sera were added. Bound antibody was detected with a peroxidase-labeled anti-human IgG and TMB substrate. The plates were read at 450 nm, and the endpoint titers were interpolated from the linear portion of the titration curve, yielding a mean absorbance of 0.5 optical density units. For indeterminate serum samples, an inhibition assay was performed. In this assay, the patient sera were pre-incubated with individual synthetic GWGR or GPGR peptides, and the percentage inhibition of antibody binding to coated wells was calculated for GWGR- and GPGR-specific peptides. Inhibition greater than 50% was used as the cutoff for defining V3 serotype for these samples. The use of V3 serology to discriminate subtype B GPGR and GWGR strains in Brazil has previously been validated by DNA sequence–typing.[15]

Definitions and statistical methods

AIDS-defining events were determined in accordance with guidelines of the Centers for Disease Control and Prevention.[18] Differences in patient characteristics or laboratory values of the B'-GWGR and B-GPGR groups were tested for statistical significance with Yates' corrected χ^2 for proportions or the non-parametric Kruskal-Wallis test for continuous variables. Follow-up time was defined from the date of entry into the cohort until the occurrence of an AIDS-defining event, or patients were censored at the beginning of combined ART or on the date of their most recent $CD4^+$ T-cell count. Patients were excluded from the current analyses if, at the time of entry into the cohort, they had had an AIDS-defining condition, primary HIV infection, or previous combined ART. The incidence of AIDS-defining events was calculated from the number of events which occurred per person-year of follow-up for all strata. Risk ratios and their 95% confidence intervals were calculated using Epi Info (version 6.04c; CDC, Atlanta, GA, USA). Adjusted hazard ratios and their 95% confidence intervals were derived from Cox proportional hazards models using STATA software.[19] Survival curves for the time to AIDS-defining events among the GWGR and GPGR groups were plotted using Graph Pad software (version 1.0, Prism, San Diego, CA, USA). The log-rank statistic was calculated to test for equality of survivor functions.

RESULTS

Between October 1987 and December 2000, 219 HIV-1-infected patients consented to participate in the cohort. V3 serotyping was performed for 191 patients: 41 (22%) patient sera reacted with peptides designed from the Brazilian subtype B strain (B'-GWGR motif), 125 (68%) samples reacted with peptides designed from the US/European subtype B strain (B-GPGR motif), and for 25 (11%) samples, serotyping was inconclusive. In 28 (13%) subjects, serum samples were not available for V3 serotyping.

Among the 166 patients in the cohort with definitive V3 serology, 42 patients were excluded because they had been symptomatic upon entry into the cohort: 40 presented with AIDS or ARC, and two cases had other illnesses which precluded them from entry into the analysis. Among the 124 subjects who met the inclusion criteria for this analysis, nine had received combined

ART before entry into the cohort and were excluded from analysis, and one patient had a missing T CD4+ cell count at entry. Thus, 114 asymptomatic patients with no prior combined ART were included in the analysis.

The characteristics of the patients included in the study are shown in Table 1. Based on V3 serology, 91 (80%) patients were classified as B-GPGR and 23 (20%) as B′-GWGR. Proportions of male gender, median age, mode of transmission, pretreatment T CD4+ count and pretreatment plasma HIV RNA levels were not significantly different between the two groups. Although the median T CD4+ cell counts at entry were not significantly different, a higher proportion of patients in the B′-GWGR group had CD4+ counts less than 200 cells/mm^3. The self-reported route of HIV transmission for the sample included 55 (48%) men who have sex with men, 40 (36%) individuals who reported heterosexual contact, 3 (2%) intravenous drug users (IDUs) and 16 (14%) patients who reported unknown or no risk factor. PCP prophylaxis was given to all patients who presented CD4+ T-cell counts below 200 cells/mm^3, and azidovudine (AZT) or didanosine (DDI) monotherapy was given to 37% and 38% of GWGR and GPGR individuals in this cohort, respectively. The types of AIDS-defining illnesses are shown in Table 1. The incidence of AIDS-defining events in the sample was not significantly higher among men or among patients less than 35 years of age (Table 2). Patients with T CD4+ cell counts less than 200 cells/mm^3 or 200–400 cells/mm^3 experienced an increased incidence of AIDS-defining events (RR: 5.71; 95% CI 1.81–17.9) compared with patients who entered the cohort with T CD4+ cell counts greater than 400 cells/mm^3; (RR: 3.11; 95% CI 1.04–9.24). Two AIDS-defining events occurred among 23 HIV-1-infected subjects with B′-GWGR serology during 68.3 person-years of follow-up (both patients had CD4+ counts <200 cells/μL), versus 16 events among 91 B-GPGR individuals during 232.7 person-years of follow-up (3 of 16 had CD4+ counts <200 cells/μL). No AIDS-defining events occurred among B′-GWGR patients with T CD4+ cell counts above 200 cells/mm^3 at entry, whereas B-GPGR patients with T CD4+ cell counts of 200–400 or above 400 cells/mm^3 presented six and seven AIDS-defining events, respectively. The survival times to AIDS-defining events among patients with B′-GWGR or B-GPGR serology are shown in

Table 1. Characteristics at baseline and follow-up of 114 asymptomatic HIV-1-infected patients

Characteristics	*GWGR n=23 (%)*	*GPGR n=91 (%)*	*p value*
Gender: n (%)			
Female	9 (39)	32 (35)	0.9
Male	14 (61)	59 (65)	
Age: mean years ± SD	37±8	37±7	1.0
Risk: n (%)			
Homo/bisexual men	11 (48)	44 (48)	0.9
Heterosexual	9 (39)	31 (34)	
Intravenous drug users	0 (0)	3 (3)	
Unknown, others	3 (13)	13 (14)	
T CD4+ cells/mm^3 at entry: n (%)			
<200	7 (30)	9 (10)	0.04
200–400	5 (22)	31 (34)	
>400	11 (48)	51 (56)	
T CD4+ cells/mm^3 (median (range)			
Entry	382 (22–1755)	446 (47–441)	0.7
Prior to initiation of HAART	241 (30–1021)	297 (29–738)	0.8
Plasma HIV RNA , $\log_{10}$ s/mL (median ± range)			
Prior to initiation of HAART	4.72 (2.5–5.9)	4.6 (1.6–6.08)	0.9
AIDS-defining events[a]	2 (9%)	16 (18%)	0.5

[a]AIDS-defining events (according to CDC, 1997) which occurred among HIV-1-infected individuals who started as asymptomatic: B′-GWGR 1 *P. carinii* pneunomia (PCP) and 1 disseminated herpes zoster (more than two dermatomes); B-GPGR: 6 oroesophagus moniliasis; 4 pulmonary and extrapulmonary tuberculosis; 2 disseminated herpes zoster; 1 encephalitis toxoplasmosis, 1 chronic diarrhea by *Cryptosporidium* sp., 1 cerebral cryptococcocis, 1 PCP.

Table 2. Incidence of AIDS-defining events among 114 initially asymptomatic subjects with seroreactivity to HIV-1 subtype B GWGR or GPGR peptides

Variable	*N*	*Follow-up time (person-years)*	*Number of events*	*Incidence rate/ person-year*	*Risk ratio (95% C.I.)*	*Adjusted hazard ratio[a] (95% C.I.)*
Gender						
Men	73	164.9	14	0.085	2.89 (0.95–8.78)	3.11 (0.93–10.35)
Women	41	136.1	4	0.029	Reference	
T CD4 count						
<200	16	26.9	5	0.186	5.71 (1.81–17.9)	8.29 (2.48–27.69)
200–400	36	59.3	6	0.101	3.11 (1.04–9.24)	3.80 (1.20–12.02)
>400	62	214.8	7	0.033	Reference	
Age						
<35	54	118.8	9	0.076	1.53 (0.61–3.86)	1.45 (0.57–3.65)
≥35	60	182.2	9	0.049	Reference	
V3 serotype						
GWGR	23	68.3	2	0.029	0.43 (0.09–1.85)	0.16 (0.03–0.93)
GPGR	91	232.7	16	0.069	Reference	

[a]Adjusted in Cox proportional hazard model for gender, age (<35 years or ≥35 years), T CD4+ cell count at the time of entry into the cohort (<200, 200–400, ≥400 cells/mm^3) and variant of HIV-1 subtype B.

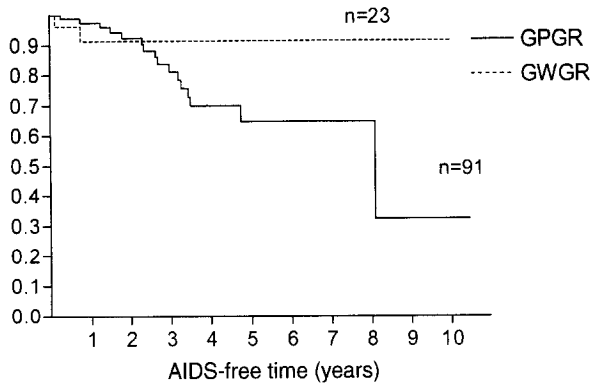

Figure 1. Time to AIDS-defining events prior to antiretroviral therapy for 114 asymptomatic patients infected with GPGR or GWGR variants of HIV-1 subtype B.

Figure 1. In a proportional hazard model including age, gender, T CD4+ cell count at entry into the cohort, and V3 serology, B′-GWGR serology was associated with decreased hazard for presenting an AIDS-defining condition during follow-up (adjusted hazard ratio 0.16; 95% CI 0.03– 0.93), while patients with decreased T CD4+ cell counts at entry were at increased risk (Table 2). During follow-up, three patients in the sample with B-GPGR serology died after experiencing an AIDS-defining event. None of the patients with B′-GWGR serology died during follow-up.

The B′-GWGR patients experienced only two AIDS-defining events, despite 68.3 person-years of follow up. If the rate of progression to AIDS-defining events for the B′-GWGR group been equal to that of the B-GPGR group (0.069 events/person-years), we would have expected five AIDS-defining events in the B′-GWGR group during the follow-up time.

DISCUSSION

The present study suggests that HIV-1 infected patients with seroreactivity to the Brazilian subtype B variant (B′-GWGR) have a decreased rate of progression to an AIDS-defining illness compared to those with B-GPGR serology. This decreased risk was found to be independent of T CD4+ cell count in a proportional hazards model. T CD4+ cell count was the most important predictor of disease progression in univariate analyses. Although a small number of AIDS-defining events were observed in patients with B′-GWGR serology, the observation of different rates per person-year of follow-up time after adjusting for T CD4+ cell count suggests that there is a biological difference between the infecting subtypes.

The current study reinforces the findings of an investigation of clinical outcomes in patients with subtype B′-GWGR or B-GPGR serology in a cohort of HIV-1-infected individuals in Rio de Janeiro, Brazil.[16] Among 321 patients in the cohort from Rio, 32% of B-GPGR-reactive individuals versus 22% of B′-GWGR-reactive patients developed AIDS-defining events, and the B′-GWGR group had decreased risk of progression to AIDS-defining events in a proportional hazards model including age and mode of acquisition of HIV.[16] These investigators included follow-up time when ART was received, because the proportion of patients in each group who received ART did not differ. In the present study, follow-up time after the initiation of ART was not included in the analysis, because the hazard of AIDS-defining events is influenced by therapy. In the present cohort in São Paulo, V3 serology was not found to be associated with younger age, in contrast with the cohort in Rio de Janeiro, in which younger patients showed more B-GPGR reactivity and a higher proportion of AIDS-defining events.[16] Although the consistency of the findings from these two studies in Brazil supports the hypothesis of a direct effect of the subtype B variant (B′-GWGR), the possibility exists that an as-yet unidentified cofactor accounts for the observed differences. The current study did not have adequate power to investigate additional risk factors for disease progression.

Fortunately, patients infected with either B′-GWGR or B-GPGR strains showed similar responses to anti-HIV therapy after the initiation of treatment, once ART became available in Brazil and the patients decided to begin therapy (data not shown). This demonstrates that reverse transcriptase (RT) and protease genes are conserved, allowing for a good response. Even greater genetic divergence than that between these two subtype B strains, such as that between subtypes B and C, was not shown to influence either the natural history or the response to HAART in a report from Israel.[20]

We do not have an explanation for the decreased pathogenicity of the B′-GWGR V3 serotype. It has been shown that some HIV subtypes, such as subtype D, possess greater ability to induce sincitia, indicating a more aggressive biological phenotype.[21] One good example of how changes in viral structure may interfere with pathogenesis is the HIV strain with a deleted *nef* gene.[1] However, this strain was passed to several individuals through unscreened blood transfusion, and did not encounter host mucosal immunity, suggesting that it has not emerged following viral adaptation and selection. In patients infected with this strain, in spite of more a stable trend than in individuals infected with wild-type HIV-1 virus, T CD4+ cell count has been decreasing, showing that nef- HIV-1 is not completely attenuated but may show a different rate of progression to disease in long-term follow-up.[22]

In contrast, the Brazilian subtype B variant (B′-GWGR motif) is widely disseminated and passed through several modes of transmission. The possibility that subtype B variant GWGR emerged recently from a viral adaptation of the GPGR motif is highly improbable, since mutations causing amino acid changes are rare. Intermediary mutation in the tip of V3 loop, such as CCA to TGG, has hardly ever been described in Brazil (or only once?).[23] Therefore, the B′-GWGR strain has been subjected to selective pressures to be main-

tained as an important strain in Brazil. It may be evidence of a founder effect rather than a better viral adaptation. However, if the Brazilian B′-GWGR variant has been less virulent than the US/European strain B-GPGR, this may represent improved selection for dissemination, and we would expect its prevalence among HIV-1-infected individuals in Brazil to increase through time. In addition, some countries close to Brazil, such as Bolivia and Chile, have reported 10% prevalence of the B′-GWGR strain, indicating either a connection with the Brazilian epidemic or later introduction of the variant into the population.[24,25] In Trinidad and Tobago, a different subtype B variant strain with a unique signature in the V3 loop, deletion of one threonine just C-terminal of the crown of the loop, has been present without increasing in prevalence since the beginning of the HIV-1 epidemic.[26]

One source of misclassification in the present study could be that the EIA with synthetic peptides is not able to distinguish all of the V3 diversity in Brazil. In fact, HIV-1 isolates have shown extensive variability in the V3 region. For this reason, we have only analyzed samples with distinct GWGR/GPGR serologic profiles. Also, subtype F infections may account for some GPGR reactivity, but subtype F represents less than 10% of HIV-1 infections in São Paulo, and the GWGR motif has not been identified in the V3 region of subtype F strains.[5]

The GWGR/GPGR serology was observed to be stable in the same patient over time in repeated assays performed during this investigation. One hypothesis had been that the presence of quasi-species could interfere with the ability of the EIA to distinguish the Brazilian subtype B variant (B′-GWGR) from the US/European variant. We noted that individual patients maintained GWGR seroreactivity over time, and serial serum samples did not bind to B-GPGR synthetic peptides (data not shown). In fact, HIV-1 subtype B quasi-species in a naive population appear to evolve though unbiased expansion around a stationary consensus sequence. This indicates that the sampling moment, relative to the seroconversion data, will not greatly influence the results of phylogenetic analysis.[27]

A biological explanation is needed for the observed decreased pathogenesis of the Brazilian variant subtype B strain with the GWGR motif when compared to that with the GPGR motif in the V3 loop region. The influence of these amino acid substitutions in the viral envelope upon clinical outcomes may be a model for investigating the role of the envelope in the natural history of HIV infection.

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