Trends in Antiretroviral Drug Resistance and Clade Distributions Among HIV-1–Infected Blood Donors in Sao Paulo, Brazil

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Background: We analyzed rates of drug resistance mutations in antiretroviral-naive São Paulo blood donors with recently acquired or established HIV-1 infections and characterized clade diversity in this population.

Methods: Six hundred forty-eight seropositive blood donor specimens were identified at the Blood Center of São Paulo between July 1998 and March 2002. To discriminate recent infections, samples were subjected to the standardized testing algorithm for recent HIV seroconversion (less-sensitive enzyme immunoassay) testing algorithm. There were 531 samples with a sufficient volume of serum to attempt polymerase chain reaction (PCR) and viral sequencing; 341 (64%) samples yielded a PCR product that could be sequenced for the reverse transcriptase and protease genes. Mutations were analyzed using the 2005 International AIDS Society mutation list.

Results: Of 341 specimens successfully analyzed, 21 (6.3%; 95% confidence interval [CI]: 3.9% to 9.3%) had drug-resistant mutations. The proportion of resistant strains was 12.7% (95% CI: 5.2% to 24.5%) among recently infected individuals compared with 5.0% (95% CI: 2.8% to 8.2%) among those with long-standing infections ($P = 0.03$). No change in the proportion of drug-resistant strains was observed among recently infected donor samples from the first half of the study period (4 of 32 samples) as compared with the second half (3 of 23 samples; $P = 0.95$). Of the 341 samples, 277 (81.2%) were classified as subtype B, 25 (7.3%) as subtype F1, 13 (3.8%) as subtype C, and 26 (7.6%) as recombinant strains. The distribution of HIV-1 subtypes was similar among recent and long-standing infected individuals and over time.

Conclusions: The prevalence of drug-resistant mutations among newly diagnosed persons in São Paulo city is low and similar to what has been described in Europe and the United States. Although HIV-1 subtype B remains predominant, subtypes F and C and recombinant forms are present in substantial proportions in infected donors.

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In Brazil, health care is provided by the state to all HIV-infected individuals. In 1996, a law was enacted guaranteeing free access to antiretroviral therapy for all HIV-infected Brazilians who required treatment, according to Brazilian guidelines.[1] The widespread use of antiretroviral drugs has led to a decline in AIDS-related mortality in Brazil.[1] It is expected that the proportion of patients experiencing virologic failure, and consequently harboring resistant strains of HIV, will increase over time.[2]

There are limited studies evaluating the prevalence of drug-resistant virus among recently infected individuals in Brazil.[3,4] Our objective was to evaluate the trends in the prevalence of HIV-1 drug resistance mutations and the distribution of HIV subtypes or clades among São Paulo city blood donors with recently acquired versus long-standing HIV infection as determined using the standardized testing algorithm for recent HIV seroconversion (STARHS) approach developed by the Centers for Disease Control and Prevention (CDC).[5] São Paulo city has the highest number of AIDS cases and is considered the epicenter of the HIV epidemic in Brazil; hence, characteristics of the virus observed among newly diagnosed blood donors likely reflect and presage national trends.

METHODS

Subjects

In Brazil, blood collection programs follow similar rules to those in the United States, including preclusion of paid donations and exclusion of prospective donors who acknowledge risk factors for HIV infection. During the period from July 1998 to March 2002, a total of 648 blood donors at the Blood Center of São Paulo (Fundação Pró-Sangue/Hemocentro de São Paulo) were identified as HIV-seropositive based on enzyme immunoassay (EIA) and Western blot results.[6] Of the 648 infected subjects, a sufficient volume of serum was available for 531 samples for evaluation in the present study. A database was created associating the samples with epidemiologic data, including gender, age, year of donation, and type of

donation. The recoded samples and epidemiologic data were then unlinked from the unique donor identifiers before further testing. This anonymized study protocol was approved by Brazilian and University of California, San Francisco ethics committees.

Determination of Recent Infection Status

To distinguish recently infected individuals from those with long-standing infections, we used the STARHS approach (Vironostika HIV-1 EIA; bioMerieux Industry, Raleigh, NC.[7] This testing was performed on 525 of the 531 confirmed HIV-seropositive specimens at the Blood Systems Research Institute (BSRI; San Francisco, CA); for 6 samples for which sequence data were obtained, we could not perform the STARHS testing because of an inadequate volume of serum in tubes sent to the BSRI.

Amplification and Sequencing

A nested polymerase chain reaction (PCR) was used to obtain 1 fragment containing the entire protease (PR) gene and approximately 700 base pairs of the reverse transcriptase (RT) gene. The primers K1/K2 were used in the first-round amplifications,[8] and the primers DP10 and F2 were used in the second-round amplifications.[9] A total of 341 of 531 samples yielded appropriately sized amplicons. To obtain sequence results for the entire amplified segment in both strands, we used at least 6 primers for sequencing each sample, including F1, F2, DP10, and DP11 primers and a new pair of primers: GABO 1 (sense-5′-CTC ARG ACT TYT GGG AAG TTC-3′) and GABO-2 (antisense-5′-GCA TCH CCC ACA TCY AGT ACT G-3′). Sequence data were obtained using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied BioSystems, Foster City, CA) according to manufacturer's protocol in an automated sequencer (ABI 377 Sequencer; Applied Biosystems).

Subtype Analysis

To define the subtype of each strain, we used interface software that automatically submits the sequences to 2 programs: recombinant identification program (RIP)[10] and basic local alignment tool (BLAST)[11] In the RIP program, the sequence is "cut" into fragments of 200 base pairs in 1 base-pair steps. Each fragment is compared with a set of reference strains, and for each fragment, the program determines which reference sequence has the highest similarity rate to that fragment. In the Blast approach, the sequence is cut into nonoverlapping fragments of 200 base pairs. Each fragment is submitted to a bank of 10,000 sequences previously subtyped in the Los Alamos databank. The program detects which databank sequence is most similar to each fragment. If the results of both analyses are in agreement, the sample is considered subtyped. Recombinant strains and sequences that did not yield concordant subtype results were manually reviewed using the bootscanning technique in SIMPLOT.[12]

Drug Resistance Analysis

All samples that contained at least 1 major mutation included in the 2005 International AIDS Society list were considered resistant.[13] We have also included any mutation at position 215 of the RT.

GenBank accession numbers for these sequences are AY999349–AY999681 and DQ003015–DQ003022.

Statistical Analysis

The prevalence of HIV resistance mutations and HIV clades was estimated as the proportion of samples successfully tested with specific resistance mutations or different clades. The χ^2 and Fisher exact tests for expected cell sizes of 5 or less were used to compare proportions between those with recent compared with long-standing HIV infection. Logistic regression was used to assess differences in prevalence of resistance strains by period of blood collection (first period from July 1998 to May 2000 and second period from June 2000 to March 2002), subtype, and STARHS results. Statistic analysis was performed using Stata Software.[14]

RESULTS

Of the 531 HIV-infected donor samples evaluated, 341 (64.5%) could be amplified and sequenced for RT/PR genes enabling drug resistance and subtype classification. There was no difference in the proportions of samples successfully amplified and sequenced when analyzed by year of collection or available demographic characteristics (eg, gender, age). Furthermore, similar proportions of samples were characterized for resistance profile and subtype from donors with recent (55 [61.8%] of 89 donors) versus long-standing (280 [64.4%] of 435 donors) infection status.

Of the 341 samples evaluated, 21 (6.1%; 95% confidence interval [CI]: 3.9% to 9.3%) had 1 or more mutation(s) associated with drug resistance. Of these, 12 samples had a mutation conferring resistance to nucleoside reverse transcriptase inhibitors (NRTIs), 3 to nonnucleoside reverse transcriptase inhibitors (NNRTIs), and 4 to protease inhibitors (PIs). Two samples contained isolates with dual-class (1 NRTI/NNRTI and NRTI/PI each) resistance (Table 1).

The proportion of strains with evidence of resistance was significantly higher among donors with recently acquired infections (12.7%; 95% CI: 5.2% to 24.5%) compared with newly diagnosed donors with long-standing infections (5.0%; 95% CI: 2.8% to 8.2%) ($P = 0.03$ in the univariate analysis and $P = 0.02$ in the multivariate analysis; Table 2). The proportion of drug-resistant strains was higher among non-B viruses ($P = 0.05$ by multivariate analysis).

The frequency of drug resistance was not associated with the period of collection when all cases were analyzed or in an analysis restricted to recently acquired infections (see Table 2). Specifically, for the 55 recently infected individuals, the proportion of resistant strains was similar in the first half of the study period (4 of 32 subjects) as compared with the second half of the study period (3 of 23 subjects) ($P = 0.95$).

Of the 341 samples evaluated, 277 (81.2%) were classified as subtype B, 25 (7.3%) as subtype F1, 13 (3.8%) as subtype C, and 26 (7.6%) as recombinant strains (23 as B/F1, 2 as B/C, and 1 as CRF_02 AG). The frequency of non-B strains was similar among recent (10.1%) and long-standing

TABLE 1. Mutation Patterns Found in the Viral Sequences From the 21 Samples With Resistant Genotypes

Sample No.	Mutations at PR Gene*	Mutations at RT Gene*	STAHRS Status	Subtype	Drugs
143	M36I, L63S	V108I/V	Long standing	B	NNRTI
228		M41L	Long standing	B	NRTI
235	L10I, M46I, L63V		Long standing	B	PI
256	I93L	V75A/V, V118I	Long standing	C	NRTI
282	M36I, M46L		Long standing	F1	PI
327	L10I, M36I, L63S	M184M/V	Long standing	B	NRTI
357	L10I, M36I, L63T, I93L	Y181C, L210S, K238R	Long standing	B	NNRTI
399	K20R, M36I	T215S	Long standing	F1	NRTI
417	L10V, M36I	T215F/S	Long standing	F1	NRTI
438	M36I	T215I/T	Long standing	F1	NRTI
468		M41L, T215L	Long standing	B	NRTI
501		M41L, K101N, K103N, M184V, T215Y	Long standing	B	NRTI + NNRTI
550	L10V, M36I, L63A	K101R, K103K/N	Long standing	B	NNRTI
575	M36I, I93L	E44D, A62V	Long standing	BF1	NRTI
67	K20R, M36I, M46L, V82A, I93L	M41L, M184V	Recent	F1	PI + NRTI
205	L63P	A62V	Recent	B	NRTI
238	M46L, L63P	V118IV	Recent	B	PI
332	M46L, L63H		Recent	B	PI
402	L63P, I93L	T69N, K70R	Recent	B	NRTI
542	L63P	M41L, E44D, D67G, T69D, Q151M, T215Y	Recent	B	NRTI
577	L63P	M41L, T215D	Recent	B	NRTI

*Mutations used in the International AIDS Society score are underlined.

infections (20.7%) ($P = 0.09$) as well as in the first half compared with the second half of the study (35 of 172 samples vs. 29 of 169 samples; $P = 0.45$).

DISCUSSION

In this study, we analyzed primary drug resistance and HIV-1 clade distributions for 341 HIV-infected blood donors diagnosed in São Paulo, Brazil from July 1998 through March 2002. Based on the STARHS algorithm, 55 (16%) of the 341 donors yielding analyzable sequence data were classified as recently infected individuals. The proportions of recent infections and other demographic characteristics were similar among those donors who were successfully analyzed compared with the nonanalyzable donors. The low amplification rate was probably attributable to the fact that the samples were obtained after serologic screening and confirmatory testing, and hence were not optimally stored for future PCR analysis. This issue was unlikely to have biased our findings. The other possible bias in our study is the fact that the STARHS assay we used has not been fully validated for subtype F. Longer window periods (ie, time from seroconversion by the sensitive to less-sensitive EIA) have been reported to occur for some for non-B samples. If this was were a confounder, we would have expected a higher rate of non-B strains among recently infected individuals, whereas our results were in the opposite direction, with a (nonsignificantly) higher prevalence of clade B samples among recently infected individuals ($P = 0.09$). A high proportion of the non-B samples were recombinant B/F or B/C strains, and it seems that the antibody response measured using the STARHS approach is similar for these strains and pure B strains.

We did not detect a trend toward increasing non-B subtypes over time. This could be because of the relatively short length of the study period or because the distribution of

TABLE 2. Distribution of Antiretroviral Drug-Resistant Strains According to Duration of Infection, Subtype, and Period of Blood Collection

	Number of Resistant Strains/Total Analyzed	Frequency (%)	Unadjusted Odds Ratio	95% CI	*P*	Adjusted Odds Ratio	95% CI	*P*
Long-standing infection	14/280	5.00	1					
Recently infected	7/55	12.73	2.77	1.05 to 7.28	0.03	3.23	1.21 to 8.68	0.02
Subtype B	14/277	5.05	1					
Subtype non-B	7/64	10.94	2.3	0.88 to 6.01	0.08	2.66	0.99 to 7.08	0.05
First period*	10/170	5.88	1					
Second period†	11/165	6.67	1.2	0.47 to 2.73	0.77	1.26	0.51 to 3.10	0.62

*From July 1998 to May 2000.
†From June 2000 to March 2002.

HIV subtypes has stabilized in Brazil. The fact that the prevalence of subtype B among donors with recent infections was higher than among those with long-standing infections suggests that the HIV subtype distribution in the city of São Paulo is relatively stable.

The overall prevalence of drug-resistant mutations was low at 6.3% (95% CI: 3.9% to 9.3%). Nevertheless, the prevalence of resistance in donors with recent infections was 12.7% (95% CI: 5.2% to 24.5%), which is significantly higher than the 5.0% (95% CI: 2.8% to 8.2%) rate of drug-resistant virus among donors with long-standing infections ($P = 0.03$). This finding suggests that the rate of transmission of drug-resistant virus to drug-naive individuals may be increasing. Another explanation for this finding is that resistance mutations may be transmitted but revert to wild type in individuals who are not receiving antiretroviral therapy; in that case, the prevalence of resistant strains would be lower among persons with long-standing infections compared with recently infected persons and unrelated to a change in the rate of transmission of resistant viruses. One recent study, however, suggests that reversion of drug resistance among recently infected individuals may be limited.[15] We could not detect an increase in the proportion of drug resistance in the second half compared with the first half of the study period using an analysis of the recently infected subset or of the total group of infected donors. We have found that drug resistance was higher among individuals infected with non-B strains. One possible explanation for this finding is that that individuals infected with non-B strains represent specific risk exposure groups (eg, injection drug use, sexual networks) in which drug resistance, and thus secondary transmission of resistant strains, is more common.

The primary drug resistance rates observed in this study are similar to results from the United States, Canada, and Europe[2] as well as to recent data from high-risk populations in Brazil. For example, Brindeiro and colleagues[4] evaluated 409 long-standing drug-naive individuals collected from 8 different sites in Brazil and found 5.4% drug-resistant strains.

The decision in Brazil to distribute free antiretroviral medication to low-income populations has raised the concern that development and transmission of resistance strains could increase rapidly because of poor compliance. Our data demonstrating a low and stable rate of primary drug resistance in newly diagnosed blood donors are reassuring in this regard. Nonetheless, we believe that continued characterization of rates of primary drug resistance as well as clade distributions among infected donors is warranted in Brazil and other developing countries to monitor for transmission of viral variants in this large and demographically diverse population.

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