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Sequence Note

HIV Type 1 Subtypes in Argentina and Genetic Heterogeneity of the V3 Region

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In the Americas outside of the United States, HIV-1 infection was originally documented predominantly among homosexual/bisexual men who initially had sexual relations with individuals from the United States. Subsequently, indigenous transmission evolved.[1] The increase in HIV infection in Latin America and the Caribbean is primarily attributable to heterosexual transmission of the virus. However, surveillance data show that drug injectors represent a rapidly growing proportion of AIDS cases in Argentina and Brazil. Drug injection has become the second largest HIV transmission category in both countries.[2] A total of 7437 cumulative AIDS cases was reported in Argentina (as of March 31, 1995), with a cumulative attack rate of 22.5 cases per 100,000 inhabitants.[3] Between 1986 and the end of July 1995, 1270 AIDS cases were reported in the province of Santa Fe (population 2.8×10^6). Rosario (population 1.2×10^6), the major city of Santa Fe, accounts for 86.3% of the AIDS cases in the province (4 times more cases per 100,000 inhabitants than the general rate of the country), of which 65% were intravenous drug users.[4]

In this study we examined the genetic variation of HIV-1 strains circulating in Rosario. Twenty-four HIV-1-infected individuals having different risk activities (Table 1) and visiting the Center of Technology and Public Health (National University of Rosario, Rosario, Argentina) in April 1995 were enrolled in this study (Table 1). From each individual DNA was extracted from 100 μl of whole blood, and the polymerase chain reaction (PCR) was performed for heteroduplex mobility assay (HMA)[5] purposes. An additional PCR round was introduced in order to obtain a sufficient amount of PCR fragment to perform an adequate HMA. The HMA resulted in HIV-1 subtype classification of 21 samples as subtypes B (n = 18) and F (n = 3). For the remaining three specimens (23, 33, and 56) and for eight samples belonging to subtype B and three samples belonging to subtype F by HMA, a 250-base pair (bp) fragment encoding the Env C2V3 region was directly sequenced and analyzed. The 14 newly determined HIV-1 *env* sequences were aligned with 19 previously known sequences of HIV-1 isolates of different geographic origin and the sequence of the HIV-1-related chimpanzee isolate SIV_{cpzgab} on the basis of primary structure. Distance calculation, tree construction, and bootstrap analysis were realized with the software package TREECON as previously described.[6] AR21 was not included because of its short sequence. In the tree, shown in Fig. 1, 10 of 14 Argentinian specimens (AR6, AR13, AR22, AR23, AR24, AR29, AR33, AR34, AR35, and AR56) clustered with members of subtype B, supported by 970 of 1000 bootstrap trees. Three of 14 Argentinian specimens clustered with subtype F strains, supported by 99.6% of the bootstrap trees. For Argentinian specimens belonging to subtype B, interhost distances at the nucleotide level were on average 11.7%; 8.3% (between AR13 and AR22) to 19.9% (between AR23 and AR56). For Argentinian specimens belonging to subtype F, interhost distances were on average 11.7%; 6.6% (between AR16 and AR18) to 14.2% (between AR15 and AR16/AR18). AR15 appeared to be a subtype F/B hybrid based on sequence analysis of a 900 bp *env* fragment encoding V3, V4, V5, and the start of gp41 (G. Myers, personal communication). From the epidemiological data (Table 1) it is apparent that subtype B viruses are spread in the heterosexual and homosexual population as well as among intravenous drug users; subtype F variants circulate among heterosexual individuals, intravenous drug users, and prostitutes in Rosario.

The predicted amino acid sequence of the V3 region for these strains is presented in Fig. 2.

A broad spectrum of tetrameric amino acid sequences was observed at the apex of the V3 loop, including GPGR (n = 2), GPGK, GWGR, GFGR°, GAGR°, AGGR* (n = 2), AWGR*, APGR, and AGGK* for subtype B, and GPGR and GPGQ (n = 2) for subtype F (an asterisk [*] indicates a sequence not reported before; a degree symbol (°) denotes a sequence reported only once among 338 subtype B V3 loop apexes[7]).

In a previous study on 30 Argentinian samples taken between 1991 and 1992 from HIV-1-infected individuals having different risk activities, the V3 loop was documented.[8] Although the

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TABLE 1. EPIDEMIOLOGICAL AND CLINICAL DATA OF SUBJECTS IN STUDY

Sample	*Subtype*	*Years of infection*	*Risk activity*[a]	*CD4$^+$ count ($\times 10^6$/liter)*	*AIDS status*[a]
1	B	6	HET	274	S
2	B	8	HOM	291	S
3	B	8	HOM	368	S
5	B	1	HET	367	AS
6	B	10	IVDU	248	S
13	B	10	HET	94	S
14	B	4	HET	188	S
15	F	1	HET	604	AS
16	F	5	IVDU	622	ND
18	F	2	PROST	655	S
19	B	2	HET	754	AS
21	B	1	HET	ND	ND
22	B	5	HET	113	S
23	B	7	HET	103	S
24	B	5	HET	440	AS
27	B	7	IVDU	138	S
29	B	2	HET	641	AS
30	B	10	HET	823	AS
33	B	2	HET	604	AS
34	B	8	IVDU	216	S
35	B	8	IVDU	56	S
47	B	5	TRANSFU	102	S
51	B	2	HET	376	AS
56	B	4	IVDU	378	AS

[a]HET, Heterosexual; HOM, homosexual; IVDU, intravenous drug user; PROST, prostitute; TRANSFU, blood transfusion; S, symptomatic; AS, asymptomatic; ND, not determined.

reported V3 loop sequences were too short for phylogenetic analysis, the reported V3 consensus sequence is homologous to the consensus B sequence. An apparently lower diversity was observed at the V3 loop apex; 27 of 30 isolates had GPGR, and one each had GPGQ, GPGT, and GPGG. Warren *et al.*[9] suggested, on the basis of a serological evaluation of HIV-1-infected individuals from Argentina and the United States, using peptides in the V3 region, that a similar pool of HIV-1 subtype B isolates exists in both countries. The prevalence of HIV-1 subtype B strains is in agreement with our findings, but subtype B variants having V3 loop apexes distinct from the highly conserved GPGR motif for North American/European strains cocirculate in Rosario.

In conclusion, on the basis of this limited number of samples analyzed by HMA and/or sequence analysis, the presence of at least two HIV-1 subtypes, B ($n = 21$) and F ($n = 3$), is demonstrated in Rosario, Argentina. A similar HIV-1 subtype distribution as well as the appearance of subtype B variants having distinct V3 apexes were reported earlier for Brazil.[7] The bi-

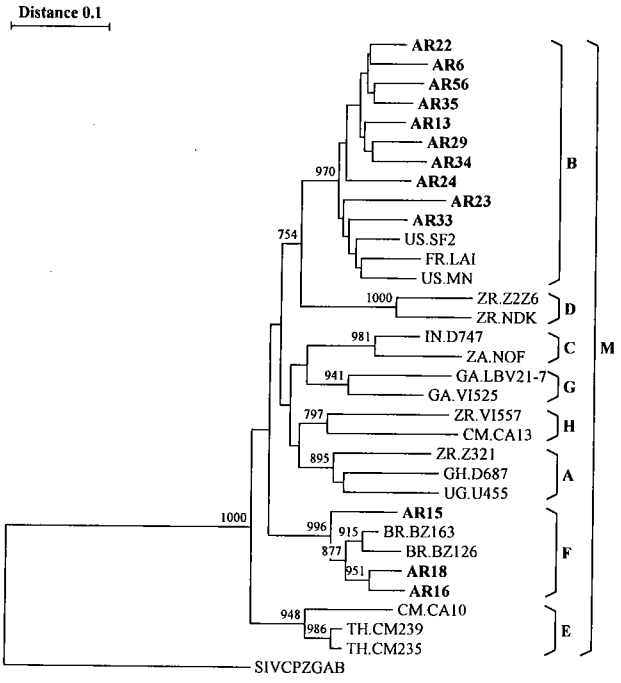

FIG. 1. Phylogenetic tree based on 407 unambiguously aligned positions of HIV-1 sequences from 32 different subjects and the simian immunodeficiency virus SIV_{cpzgab}. The sequences determined in this study are indicated in boldface. The distance between two sequences is obtained by summing the lengths of the connecting horizontal branches, using the scale on top. The number of bootstrap trees out of 1000 replications supporting a particular phylogenetic group in more than 75% is placed alongside the node considered. The nucleotide sequence data were deposited in the EMBL, GenBank, and DDBJ nucleotide sequence databases under the following accession numbers: U37030–U37043.

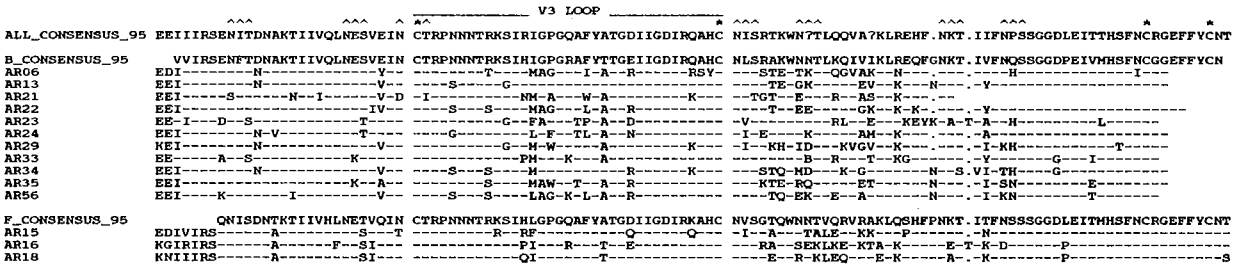

FIG. 2. Amino acid sequence alignment of Env C2V3 regions of the Argentina strains as compared to the "global" consensus, subtype B and F consensus sequences.[7] Amino acid identity between sequences is represented by dashes; points are introduced to align the sequences. (B denotes a sequence ambiguity in AR33.)

ological importance of the broadening of V3 crown diversity with regard to pathogenesis or vaccine development needs to be examined further.

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