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Subtyping of Human Immunodeficiency Virus Type 1 Strains by Using Antibodies Specific for the Third Variable Domain (V3) of gp120: Results May Be Affected by Divergent V3 Sequences

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Human immunodeficiency virus type 1 serotype C was found in 545 of 712 Ethiopian patients by peptide enzyme immunoassay. Serotyping failed in 146 samples due to the absence of V3 antibodies or multiple reactivities. In 6 of 34 samples, discordant results were obtained by serotyping and genotyping, possibly due to divergent V3 sequences.

At least nine human immunodeficiency virus type 1 (HIV-1) subtypes have been identified (7, 9). Subtyping can be performed by serotyping or genotyping. For genotyping, sequencing of *env* and/or *gag* genes has been used (9). Serotyping using V3 peptides is possible because of the difference in avidity between subtype-specific and cross-reactive antibody binding (3, 10, 13).

Sera were obtained from 712 HIV-1-seropositive Ethiopians in 1988 ($n = 143$) and 1993 ($n = 569$) and from HIV-1-infected Africans and Swedes ($n = 29$). Peptides covering the middle parts of the V3 loop were synthesized by using 9-fluorenylmethoxycarbonyl-protected amino acids (subtype A, RKSIHIG PGQAFYAT; subtype B, RKSIHIGPGRAFYTT; subtype C, RKSIRIGPGQTFYAT; subtype D, RQRTHIGLGQALYTT and RQRTHIGPGQALYTT; subtype E, RTSITIGPGQVF YRT and RKSIHLGPGQAWYTT) (8–10, 13). The purity of the peptides was >57% (mean ± standard deviation, 65% ± 8%) (11). Microtiter plates were coated with a mixture containing 0.5 μg of each peptide. Fifty microliters of each peptide (200 μg/ml) and 50 μl of serum-dilution buffer (1:50) were mixed in the plates. As a control, serum was mixed with dilution buffer. Thereafter, enzyme immunoassay (EIA) was performed as described elsewhere (13). When a peptide resulted in an optical density value of <50% of the control, and 50% less than any other peptide, the serum was classified as that particular subtype.

When significant inhibition was obtained for more than one peptide, separate wells were coated with the reactive peptides (10 μg/ml). Fifty microliters of serial dilutions (200, 100, 50, 25, and 12.5 μg/ml) of the same peptides that were used in the solid phase were incubated with 50 μl of serum-dilution buffer (1:50) in separate wells. Briefly, if a serum was inhibited with subtype A and B equally in the previous single inhibition assay, one-half of a plate was coated with peptide A (10 μg/ml) and the other half was coated with peptide B. Fifty microliters of the 200-μg/ml peptide A preparation was incubated with 50 μl of serum in wells coated with peptide A as well as peptide B. This was repeated with peptide B and with all the other peptide dilutions. The peptide giving significant inhibition at the lowest concentration in each of the wells coated with the different-subtype peptides was considered subtype specific (13). The basis for this conclusion was that the reactivity of a subtype-specific antibody is of higher avidity than that of a cross-reactive antibody. When a serum either was equally inhibited by more than one subtype-specific peptide in all tested conditions or resisted inhibition, it was categorized as nontypeable.

From Ethiopians ($n = 18$), other Africans (3 from Uganda, 2 each from Congo and Tanzania, and 1 each from Ivory Coast, Rwanda, Angola, Gambia, and Burundi), and Swedes ($n = 6$), HIV-1 DNA corresponding to p17 and C2-V3 regions was PCR amplified (1). The amplificates were directly sequenced

TABLE 1. Comparison of HIV-1 subtyping by peptide-based EIA and by V3 and p17 DNA sequencing[a]

V3/p17 genotype[b] (no. of samples)	No. of sera (%) with the following results by V3 peptide EIA:					
	HIV-1 subtype determination				Inconclusive results due to:	
	A	B	C	D	No V3 antibodies	Multiple reactivities
A/A (3)	3 (100)					
B/B (5)		5 (100)				
B/ND (1)		1 (10)				
C/C (14)			12 (80)		1 (100)	1 (20
C/ND (3)			3 (20)			
C/A (1)						1 (20)
D/D (4)		1 (10)		2 (100)		1 (20)
D/A (1)		1 (10)				
U/F (1)						1 (20)
U/G (1)						1 (20)
ND/A (2)		2 (20)				
Total (36)	3	10	15	2	1	5

[a] Sensitivity of the V3 peptide EIA versus C2-V3 genotyping, 28/34 (82%); specificity of the V3 peptide EIA versus V3 genotyping, 26/28 (93%); sensitivity of the V3 peptide EIA versus p17 genotyping, 26/32 (94%); specificity of the V3 peptide EIA versus p17 genotyping, 22/26 (85%). No samples were identified as subtype E by either method.

[b] U, unclassified or outgroup in the phylogenetic analysis; ND, not determined.

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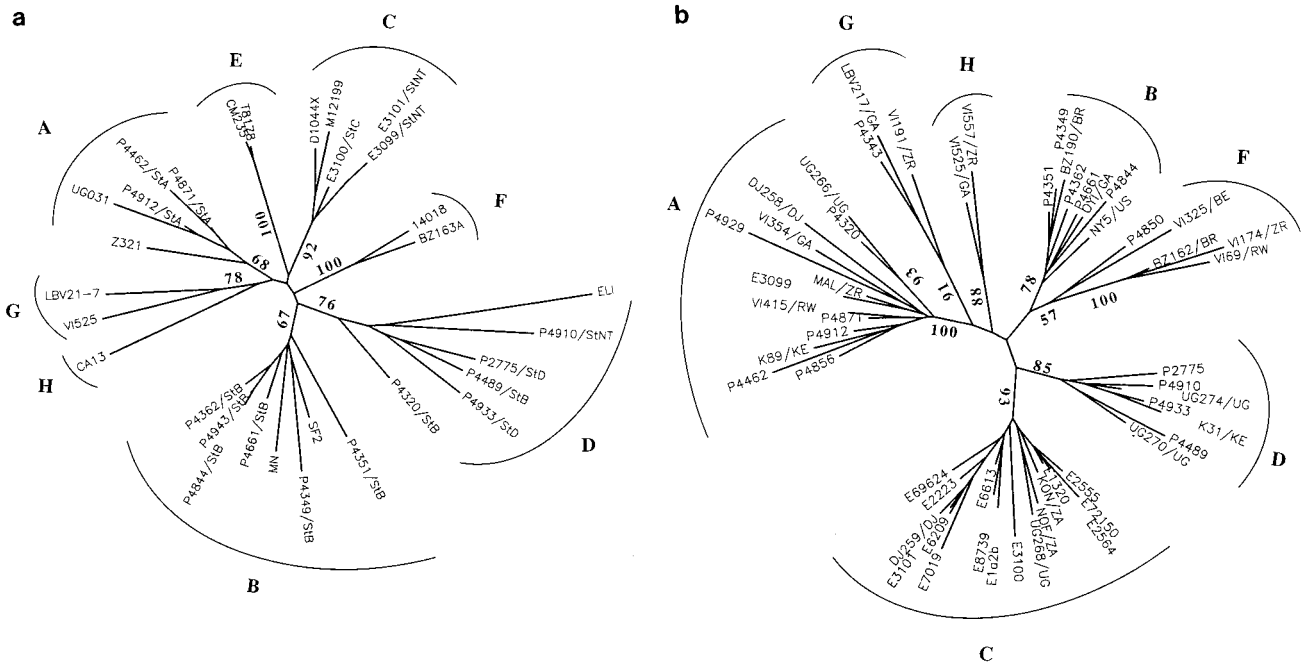

FIG. 1. Phylogenetic trees based on analysis of a 209-bp C2-V3 sequence (a) and a 250-bp p17 sequence (b). Numbers are bootstrap values obtained by sampling of 100 trees. "P" and "E" with numbers indicate the sample code, and "St" with letters stands for the serotyping result. The overall serotyping results were as follows: from Ethiopia, 18 subtype-A, 3 subtype-B, 545 subtype-C, and 146 nontypeable samples; from Sweden, 6 subtype-B samples; from other African countries, 4 subtype-A, 5 subtype-B, 5 subtype-C, 4 subtype-D, and 5 nontypeable samples.

with an automated laser fluorescent DNA sequencer (GenBank accession no. U45486-87, U45490-502, U56367-85, and U56336-66). Phylogenetic analysis was carried out with the Phylip package (version 3.5c) (4). For 30 samples, p17 and V3 sequences were obtained, of which 15 V3 sequences had been reported earlier (12). For four and two samples, only V3 or p17 sequences, respectively, were obtained.

For most samples (77%), the V3 serotyping and genotyping were concordant (Table 1). Two HIV-1 strains (E3099 and P4320) clustered discrepantly in the C2-V3 and p17 phylogenetic trees (Fig. 1). In two samples (P4343 and P4850), only the V3 sequences (109 bp) were obtained. These strains outgrouped genotypically and were nontypeable by serology. In two samples (P4489 and P4320), which exhibited V3 serotype B and C2-V3 genotype D, unusual subtype-D V3 sequences were observed (9). A Gly-Pro-Gly-Arg motif was found at positions 319 to 322, which is more common to subtype B. In two samples for which C2-V3 sequences were not obtained despite repeated attempts, subtype-A p17 sequences were found. These samples were of V3 serotype B.

The pattern of HIV-1 subtype distribution in sub-Saharan Africa is generally heterogeneous (2, 7). Countries in which HIV-1 was introduced comparatively late seem to have a homogeneous pattern (5). As herein shown, subtype C dominated in the Ethiopian epidemic, both in 1988 and 1993, suggesting that it started later than in other sub-Saharan African countries (6). Other factors may have contributed to the homogeneous subtype-C pattern. The registered prostitutes, who are major contributers to the Ethiopian epidemic, do not have much access to foreigners and therefore presumably carry mainly subtype C. In addition, most Ethiopians do not travel and the civil war restricted the accessibility of many Ethiopian regions.

A major aim of the study was to define possible reasons for multiple or erroneous reactivities in the serotype EIA. HIV-1 strains with discrepant V3 and p17 genotypes, and samples with unusual V3 sequences, gave inconclusive serotyping results. For example, HIV-1 subtype-D strains with a Gly-Pro-Gly-Arg sequence instead of the more common Gly-Pro-Gly-Gln sequence at the tip of the V3 loop were serotyped as subtype B. In contrast, two correctly serotyped subtype-D samples had two different sequences: Gly-Leu-Gly-Arg and Gly-Pro-Gly-Lys.

In conclusion, serotyping of subtype-specific antibodies to the HIV-1 V3 domain may be useful for large-scale screening and estimation of subtype distribution. However, we also identified reasons why serotyping may sometimes be misleading and thus should be interpreted with care.

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