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The Honduran Human Immunodeficiency Virus Type 1 (HIV-1) Epidemic Is Dominated by HIV-1 Subtype B as Determined by V3 Domain Sero- and Genotyping

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The distribution of subtypes A through E of human immunodeficiency virus type 1 (HIV-1) in Honduras was analyzed in 120 HIV-1 positive serum samples by V3 peptide serotyping and HIV-1 cDNA sequencing. In the Honduran HIV-1 epidemic, subtype B was detected in 98 of 99 subtyped samples.

Phylogenetic analysis of envelope sequences from human immunodeficiency virus type 1 (HIV-1) strains collected throughout the world have identified at least nine HIV-1 subtypes (8). The major subtypes are A to E, whereas subtypes F to H seem to be less prevalent (8). The subtype distribution of HIV-1 varies from continent to continent. The presence of subtype B has been documented in the United States, Brazil, Argentina, Uruguay, and Paraguay (1, 3, 6, 9–11, 13, 14). For the Central American countries, including Honduras, information on the HIV-1 epidemic is limited and no subtype distribution data is available. Characterization of local HIV-1 subtype distribution is of importance for appropriate preventive measures with respect to local transmission routes, risk behaviors, information strategies, diagnostic tests, and potential HIV-1 vaccines. Approximately 7,700 HIV-1 infections have been diagnosed in Honduras between 1985 and 1996 (7). The aim of the present study was to estimate Honduran HIV-1 subtype distribution by using 14% of the 863 HIV-1 cases sampled and diagnosed during 1994.

Serum samples from 100 HIV-1-seropositive Hondurans (54 women and 46 men; age range, 3.5 to 56 years; median age, 30 years) were obtained through the Laboratorio Central de Referencia-SIDA, Ministerio de Salud Publica, Tegucigalpa M.D.C., Honduras. The samples were obtained from all eight health districts of Honduras. The clinical statuses of the patients studied were asymptomatic (n = 49), AIDS (n = 25), AIDS-related complex (n = 21), and unknown (n = 5). An additional 20 plasma samples from HIV-1-seropositive cases were obtained from the Associacion Lucha Contra El SIDA, Tegucigalpa M.D.C., Honduras. The epidemiology of all of the patients studied is given in Table 1.

Seven 15-amino-acid-long peptides corresponding to the five major subtypes (A, B, and C, one peptide each; D and E, two peptides each) and corresponding to a part of the gp120 third variable domain (V3) were previously produced (11, 12). The peptide-based serotyping enzyme immunoassay was performed as previously described (11, 12). In brief, microtiter plates were coated with a mixture containing 0.5 μg of each peptide per ml. Prior to addition of a serum sample diluted 1:50, an equal volume of dilution buffer with or without 200 μg one subtype-specific peptide per ml was added. Thereafter, the enzyme immunoassay was performed as previously described, with semiquantitation of noninhibited antibodies (11, 12). When a subtype-specific peptide inhibited serum reactivity (optical density at 405 nm) by >50% of the noninhibited control value and 50% less than any other peptide, the sample was classified as that subtype. When significant inhibition was obtained by more than one peptide, the serum was analyzed as follows. All peptides giving significant inhibition were used at 10 μg/ml to coat separate wells on the same plate. Cross-inhibition was performed with fivefold dilutions of peptides (200 to 12.5 μg/ml) incubated together with sera on the coated plates. The peptide giving significant (>50%) inhibition at the lowest concentration (i.e., highest avidity) was considered to be subtype specific. Serum reactivities that either were equally well inhibited by more than one peptide or completely resisted peptide inhibition, were designated not typeable.

HIV-1 RNA coding for the V3 domain (346 bp) from four serum samples and nine plasma samples from the 120 HIV-1-infected Hondurans was reverse transcribed, and the cDNA was amplified by a nested PCR according to established protocols (11). The amplified products were directly sequenced with an automated laser fluorescent DNA sequencer (Pharmacia Biotech, Uppsala, Sweden).

In the initial screening, it was possible to serotype 96 (80%) of the 120 samples. Most (95 of 96) were of subtype B (Table 1). Subtype A reactivity was found in a sample from one patient from which we were unable to amplify HIV-1 RNA. Of the remaining 24 samples (20%), three (3%) were not typeable due to lack of reactivity to any of the V3 peptides and 21 (17%) were not typeable due to multiple V3 reactivities (i.e., V3 reactivities that were inhibited by more than one V3 peptide).

The V3 region was sequenced in 13 samples, and 12 sequences were sufficient in length for genotyping. Of the 13 sequenced samples, 9 were of serotype B and four were not typeable by peptide serology analysis. Sequence analysis of the V3 domain showed that a 150-bp V3 loop fragment was sufficient for correct clustering of the reference isolates (Fig. 1). The 12 Honduran V3 sequences consistently grouped in the subtype B cluster (Fig. 1). Of the 12 genotype B samples, 9 were serotyped as subtype B, indicating 75% sensitivity for the serotyping assay. Subtype B V3 sequences were found in three sera that were not typeable by serology analysis due to multiple

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TABLE 1. Distribution of HIV-1 subtypes among 120 HIV-1-infected Hondurans as determined by V3 serotyping and/or V3 cDNA sequencing

Epidemiology	No. of persons	No. with HIV-1 subtype:		No. NT[a]
		A	B	
Heterosexual	87	1	68	18
Homosexual	4	0	4	0
Prostitute	18	0	16	2
Blood donor	9	0	8	1
Child	2	0	2	0
Total	120	1	98	21

[a] NT, nontypeable.

V3 reactivities. In the fourth sample, which was obtained from a patient with a diagnosis of AIDS and was not typeable by peptides due to a lack of V3 antibodies, the V3 sequence obtained was too short for reliable analysis. This suggests that of the other Honduran samples not typeable by serology analysis, a major proportion may be of subtype B. In total, of the 98 samples with a subtype defined by serology analysis and/or sequencing, 97 were of subtype B. Serotyping alone gives a good estimation of the molecular epidemiology of HIV-1 subtypes. However, it may be insufficient to perfectly describe the panorama of a local HIV-1 epidemic.

In the present study, representing 14% of the Honduran HIV-1 cases diagnosed in 1994, HIV-1 subtype B was dominant, irrespective of geographical origin, clinical status, or risk group. This homogeneity with respect to HIV-1 subtypes is similar to the spread of HIV-1 in the western hemisphere until the late 1980s and is also similar to the present ethiopian HIV-1 subtype C epidemic (11, 12). No evidence of multiple introductions of HIV-1 in Honduras was obtained, much unlike the recent introductions of HIV-1 subtypes A, C, D, and E reported in the United States and western Europe (1, 2, 4, 5). Also, the present study shows that heterosexual contacts may be effective in spreading HIV-1 subtype B.

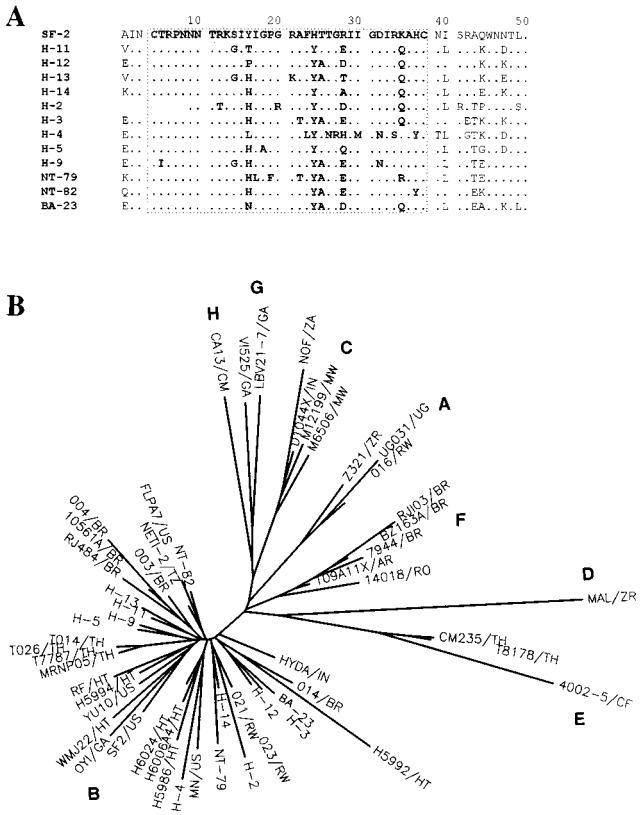

FIG. 1. (A) Alignment of HIV-1 V3 sequences obtained from nine Honduran serotype B samples (H) and three Honduran samples nontypeable by serology analysis (NT) with HIV-1 strain SF-2. (B) Phylogenetic relationships among the 12 Honduran HIV-1 V3 sequences and previously described HIV-1 V3 sequences. All analyses were performed with the PHYLIP software package.

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