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Human Immunodeficiency Virus Type 2 (HIV-2) Seroprevalence and Characterization of a Distinct HIV-2 Genetic Subtype from the Natural Range of Simian Immunodeficiency Virus-Infected Sooty Mangabeys

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The extent of zoonotic infections in rural Sierra Leone, where both feral and pet sooty mangabeys harbor divergent members of the human immunodeficiency virus type 2 (HIV-2)-sooty mangabey simian immunodeficiency virus (SIVsm) family, was tested in blood samples collected from 9,309 human subjects in 1993. Using HIV-1- and HIV-2-specific enzyme immunoassays and confirmatory Western blot analysis to test for antibodies to SIVsm-related lentiviruses, we found only nine subjects (0.096%) who tested positive for HIV: seven tested positive for HIV-1 and two tested positive for HIV-2. Compared with other rural West African communities, Sierra Leone displayed the lowest seroprevalence (0.021%) of HIV-2 infection yet reported, much lower than the previously reported seroprevalence in SIVsm-infected feral and household pet sooty mangabeys. Heteroduplex analysis demonstrated that two of the newly found HIV-1 strains belonged to subtype A, the most common HIV-1 subtype in Africa, but this is the first report of subtype A in Sierra Leone. The two HIV-2-infected individuals harbored two distinct HIV-2 strains, designated 93SL1 and 93SL2. Phylogenetic analysis indicated that HIV-2 93SL1 is a member of HIV-2 subtype A, the first strain of this HIV-2 subtype found in Sierra Leone. In contrast, HIV-2 93SL2 belongs to none of the five previously characterized HIV-2 subtypes (A to E) but is a new subtype, herein designated F, having the most divergent transmembrane sequences yet reported for HIV-2. The fact that both of the two most divergent HIV-2 subtypes known, E and F, are rare and found as single occurrences in persons from Sierra Leone may be related to the fact that this small region of West Africa also contains free-living and household pet sooty mangabeys with highly divergent variants of SIVsm. This finding provides support for the hypotheses that new HIV-2 subtypes result from independent cross-species transmission of SIVsm to the human population and that these single-occurrence transmission events had not spread widely into the population by 1993.

Ever since human immunodeficiency virus type 2 (HIV-2) was identified in West Africa in 1986 (7), its similarity with HIV-1 has been widely investigated. HIV-1 and HIV-2 share genetic and biological properties, such as genome structure, mechanisms for transactivation, and CD4 cell depletion (14, 18). Although both viruses cause immunodeficiency and AIDS in infected persons (7), HIV-2-infected persons may exhibit longer clinical latency periods (2), slower disease progression (20), and lower viral load in the asymptomatic stage (30). In addition, HIV-1 differs from HIV-2 in that HIV-2 subtypes A through E have initially been found in West Africa (11) whereas HIV-1 M subtypes are widely distributed in the world (8, 25).

The fact that HIV-1 and HIV-2 differ should not be surprising if, as has been hypothesized, they originated from genetically distant primate lentiviruses that naturally infected different simian species and diverged in them before being transmitted to humans (11, 26, 29). Supporting this hypothesis, we have shown that multiple HIV-2 subtypes could have been derived from highly divergent simian immunodeficiency virus (SIV) strains occurring in free-living and household pet sooty mangabeys (SMs) in Sierra Leone and Liberia by several independent cross-species transmission events (5). In this regard, it is important to find and characterize SIVsm-related viruses in West Africans, especially in countries where naturally SIVsm-infected SMs occur (5, 6).

All of Sierra Leone lies within the natural range of SMs (33), and this range was verified in 1993 and 1994, when we observed feral troops of SMs in all areas of that country (5, 6, 30a). However, except for two HIV-2-infected individuals identified in the United States who traveled from Sierra Leone (11, 15), HIV infection in Sierra Leone has not been characterized. Because 70% of sub-Saharan African people live outside cities, the identification and characterization of lentiviruses from rural populations is critical to understanding the natural history of HIV-2 in West Africa.

From 1991 to 1993 we observed that most persons in rural Sierra Leone had had contact with SMs (5, 6). This information, together with the finding of divergent SIVsm strains in both feral and household pet SMs in Sierra Leone (5, 6), raised further questions. Is the seroprevalence of HIV-2 in humans

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similar to that of SIV in SMs? Are the HIV-2 strains in humans residing in Sierra Leone genetically diverse? Are viruses related to SIVsm present in this rural community? What are the antigenic and phylogenetic natures of the HIV-2 strains in this area, where infected SMs are naturally found?

To answer these questions, an HIV-1 and HIV-2 seroprevalence survey of people of all ages was conducted in 1993 in a remote rural area consisting of small villages (from 9′N to the Guinea border) covering about 10,000 mi^2 (25,000 km^2) of Sierra Leone. One village from each chiefdom in the northern province of Sierra Leone was surveyed, serum samples were collected, and questionnaires on capturing and using SMs as a food source and other demographic information were prepared. The study, conducted in a rural West African setting, represents a cross section of the Sierra Leone population and therefore affords an improved view of the prevalence of HIV infection in the nonurban population. Moreover, a civil war erupted in Sierra Leone in 1991 but was confined to regions outside the study area through the time of sampling. After 1993, the war spread into this rural northern province. The serological and genetic information obtained in this study may be useful in future analysis of the war's impact on the spread of HIV-1 and HIV-2 in the region.

Here we report on 9,309 human subjects in Sierra Leone who were surveyed for HIV-1, HIV-2, and SIVsm-related lentiviruses by enzyme immunoassay (EIA) and Western blot analysis. The following results were obtained: (i) HIV-1 or HIV-2 antibody was found in only 9 of 9,309 (0.096%) specimens from this rural community; (ii) of seven HIV-1 infections, two were HIV-1 subtype A, the first subtype A viruses found in Sierra Leone; and (iii) both newly found HIV-2 strains (93SL1 and 93SL2) were members of the HIV-2–SIVsm family of viruses but were not of the same genetic subtype (HIV-2 93SL1 is a member of HIV-2 subtype A, the first subtype A to be found in Sierra Leone, and HIV-2 93SL2 is a distinct subtype, F). The single occurrence of a new HIV-2 subtype, which has not been found in other areas of Africa but has been found only in the same geographic location as SIVsm-infected SMs, provides further evidence for independent cross-species transmission of SIVsm into human beings. These data also show that this kind of cross-species transmission must be relatively rare and that the new subtype was not widespread in 1993.

MATERIALS AND METHODS

Subjects. In 1993, 9,309 individuals in 45 villages in the northern province of Sierra Leone were studied. To identify donors, each village was mapped and the houses were numbered. The study households were selected by a random-number table. Approximately 40 households or up to 250 persons were selected. In small villages all households were surveyed. The villages ranged in size from 200 to 2,000 persons. This area was chosen because free-living and household pet SMs were present and the area was a stable rural community compared to other parts of the country that had been disrupted by civil war. A numbered questionnaire was verbally administered to each person, asking about the age, medical history, education, occupation, number and type of pets, animal exposure, consumption of monkey meat, and sexual activity. Ten milliliters of whole blood was clotted from each participant, labeled with the corresponding number from the questionnaire, separated immediately, and frozen at −20°C in a field freezer. Serum specimens were shipped on dry ice and stored at −70°C until they were tested at the Aaron Diamond AIDS Research Center.

To obtain follow-up specimens for genetic analysis of HIV, the questionnaires of the nine seropositive persons were examined for demographic information. Four of the nine persons were located, heparinized blood samples were taken 9 months to 1 year after the first survey, and peripheral blood mononuclear cells (PBMC) were separated and frozen in the field. Five seropositive individuals could not be located for follow-up blood specimens due to rebel activity in the area.

EIA and Western blot analysis. All 9,309 samples were tested once by an HIV-1–HIV-2 recombinant EIA (Abbott Laboratories). Samples initially positive by the Abbott EIA were retested with Western blot strips containing an antigen to SIVsmLib1, a West African-derived SIVsm (21), and with HIV-1 and HIV-2 strips (Cambridge Biotech, Boston, Mass.). The controls were specimens from HIV-positive humans and SIVmac251-infected macaques and sera and plasma from SIVsm-infected SMs from Sierra Leone, as well as negative human, SM, and macaque sera and plasma.

PCR amplification of HIV-1 and HIV-2 gene fragments. High-molecular-weight genomic DNA from uncultured PBMC was extracted with a DNA-RNA extraction kit (United States Biochemical Corp., Cleveland, Ohio). For HIV-2, nested primers were used to amplify an 898-bp *gag* fragment covering part of the *gag* p17 and p26 regions and a 1,001-bp *env* fragment covering the transmembrane protein (gp43) region. The primers, whose entire lengths were included in both fragments, were designed both to be highly conserved among the HIV-2–SIVmac–SIVsm sequences published in the Los Alamos Database (25) and to amplify DNA fragments that allowed comparisons with other HIV-2–SIVmac–SIVsm family members. PCR was done in a model 9600 thermocycler (Perkin-Elmer, Foster City, Calif.) in 100-μl volumes containing 0.5 to 1 μg of DNA, 10 mM Tris-HCl (pH 8.5), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 mM (each) dATP, dGTP, dCTP, and dTTP, 20 pmol of each primer, and 2.5 U of *Taq* polymerase. The first round of PCR consisted of an initial cycle at 95°C for 2 min followed by 30 cycles of 95°C for 20 s, 45°C for 1.5 min, and 72°C for 2 min, with *gag* or *env* primer pair GF1 (5′-TGG GAG ATG GGC GCG AGA AAC TCC GTC-3′)-GR1 (5′-TCC ACA TTT CCA GCA GCC CTG TCT TCT-3′) or EF4 (5′-GGC TGG GAT AGT GCA GCA ACA GCA ACA G-3′)-ER1 (5′-GGG AGG GGA AGA GAA CAC TGG CCT ATA-3′), respectively. The second PCR round, with inner primer pair GF2A (5′-AGG GAA GAA AGC AGA TGA ATT AGA A-3′)-GR3 (5′-GCA TTT TGA ATC AGC AGT GTT TGA GTC ATC CA-3′) (*gag*) or EF5A (5′-TGT TGG ACG TGG TCA AGA GAC AAC-3′)-ER2A (5′-AAG CGG GAG GGG AAG AGA ACA CTG GCC-3′) (*env*), consisted of another 30 cycles of 94°C for 20 s, 55°C for 1.5 min, and 72°C for 2 min. For both reaction rounds, the last cycle's extension was at 72°C for 8 min.

To test for possible dual HIV-1–HIV-2 infections, PBMC DNA was also tested by PCR for HIV-1 and HIV-2 DNAs. In separate reactions, HIV-1 *gag* and *env* and HIV-2 *gag* fragments were amplified by nested PCRs. For HIV-1, the *gag* primer pairs were 534 and 531 for the first reaction and 535 and 531 for the second reaction (34). The *env* primer pairs were ED3-ED14 for the first PCR and ED5-ED12 for the second PCR (10). For HIV-2, the *gag* primer pairs included GF1-GR1 and GF2A-GR3 as described previously (5).

PCR amplification of HIV-1 *env* V1 through V5 gene fragments for the heteroduplex mobility assay (HMA). Nested PCR analysis of the HIV-1 *env* gene was undertaken as described previously (9). The first-round primers were ED3 (5537 to 5566) and ED14 (7509 to 7538). Two sets of second-round primers were used: ED5 (6556 to 6581) and ED12 (7822 to 7792) were used to amplify a 1.2-kb V1 through V5 region of *env*, and ES7 (7001 to 7020) and ES8 (7647 to 7667) were used to amplify a 0.7-kb V3 through V5 region of *env*. Both rounds of PCR used a reaction mix described previously (9) except that 1.25 mM $MgCl_2$ was used. First-round amplification reactions were carried out in a Perkin-Elmer thermocycler 9600 for 35 cycles under the following conditions: 3 cycles at 95°C for 1 min, 55°C for 30 s, and 72°C for 1 min; 32 cycles at 95°C for 10 s, 55°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. Second-round cycling conditions were as follows: 3 cycles at 94°C for 1 min, 55°C for 30 s, and 72°C for 40 s; 32 cycles at 94°C for 10 s, 55°C for 30 s, and 72°C for 40 s; and a final extension at 72°C for 2 min.

HMA. The use of HMA for subtyping HIV-1 strains has been described previously in detail (9). Briefly, 5 μl of the second-round PCR products from both HIV-1-seropositive persons was added to 5 μl of second-round PCR products from the analogous fragment derived from a reference strain and 1.1 μl of 10× HMA buffer (10× HMA buffer is 1 M NaCl, 100 mM Tris [pH 7.8], and 20 mM EDTA). Heteroduplexes were formed by heating the mixture to 94°C for 2 min and cooling it on ice for 10 min. The mix was then applied to a 5% polyacrylamide gel in 1× Tris-borate-EDTA buffer and electrophoresed in a V16 apparatus (Gibco BRL, Gaithersburg, Md.) at a constant 200 V for 6 h (ED5-ED12 primer pair) or 250 V for 3 h (ES7-ES8 primer pair). The DNA duplexes were viewed and photographed after ethidium bromide staining. The standard sequences used for the HMA and their corresponding GenBank accession numbers are as follows: A1 (RW20), U08794; A2 (UG37), U09127; A3 (SF170), M66533; B1 (BR20), U08797; B2 (TH14), U08801; C1 (MA959), U08453; C2 (ZM18), L22954; D1 (UG21), U08804; D3 (UG46), U08809; E1 (TH22), U09131; E2 (TH06), U08810; F1 (BZ162), L22084; F2 (BZ163), L22085; G1 (RU131), U30312; and H2 (VI557), U09666.

DNA sequencing and phylogenetic analysis. PCR products were cloned and sequenced as recently described (5). Proviral DNA sequences were aligned and analyzed as recently described (5).

Nucleotide sequence accession numbers. The nucleotide sequences obtained in this study were submitted to GenBank, and their accession numbers are U75440, U75441, and U75442.

RESULTS

HIV seroprevalence in rural Sierra Leone. Seroprevalence of HIV infection in rural northern Sierra Leone is categorized

TABLE 1. Seroprevalence in 1993 of HIV infection in persons in rural Sierra Leone where SIVsm-infected SMs occur

Gender (*n*)	Age (yr)	No. tested	Seropositive for HIV-1 (%)	Seropositive for HIV-2 (%)
Male (4,056)	<13	1,537	1 (0.065)	0
	13–19	633	0	0
	20–34	642	1 (0.16)	0
	35–49	587	2 (0.34)	0
	50 or more	657	0	0
Female (5,197)	<13	1,531	0	0
	13–19	730	0	0
	20–34	1,096	3 (0.27)	0
	35–49	1,051	0	1 (0.095)
	50 or more	789	0	1 (0.13)
Unknown (56)		56	0	0
Total		9,309	7 (0.075)	2 (0.021)

by age and gender in Table 1. After a single HIV-1–HIV-2 recombinant EIA, 167 of 9,309 (1.8%) specimens were HIV reactive. To eliminate false positives, the manufacturer of the EIA recommends repeat testing of specimens, which eliminated 35% of the first-round reactive samples. Repeat testing by Western blot analysis eliminated another 55% of the reactive samples. The expected rate for false positives is therefore almost 70% by the Abbot assay; however, by other commercial assays, rates of false positives of up to 95% have been reported for samples collected in Africa (1). In this study all 167 initially reactive samples were tested by SIVsm, HIV-1, and HIV-2 Western blotting to increase the chance of identifying minimally reactive specimens. Results of Western blot analysis, with multiple assays and kits as described in Materials and Methods, confirmed 9 of the 167 (5%) initially reactive specimens (Table 1), giving a false positive rate of about 95%. Most false positives were reactive only with p24 or a 70-kDa polypeptide. A few were negative by Western blotting. Seven contained antibodies primarily to HIV-1 antigens, and the remaining two reacted mainly with HIV-2 antigens. Therefore, in this West African rural region, the overall seroprevalence in 1993 for HIV-1 and HIV-2 infection was 0.075 and 0.021%, respectively. In questionnaires, over 60% of individuals surveyed claimed to have had contact with an SM while preparing meat as a food item or capturing or keeping SMs as pets.

Western blot analysis of plasma derived from HIV-infected subjects. For characterization of the viruses, heparinized whole blood was collected from four of the nine seropositive subjects, including both HIV-2-seropositive women. To study the antigenic relationship between the newly found HIVs and previously characterized primate lentiviruses, the four plasma samples were subjected to Western blot analysis with antigens of HIV-1, HIV-2, SIVagmTyo, SIVsmBro (5), and SIVsmLib-1 (21). Figure 1 shows the results obtained with HIV-1 and HIV-2 Western blots. The HIV-2-reactive plasma contained antibodies strongly reactive to envelope surface protein gp120, Gag precursor p58, core p26, matrix p17, transmembrane gp34 and its trimer gp105, and reverse transcriptase (RT) p68 and p55 (Fig. 1B, lanes 1 and 2). In contrast, HIV-1-infected subjects (Fig. 1A and B, lanes 3 and 4) reacted strongly with HIV-1 antigens. However, one of two HIV-2-reactive plasma samples had also reacted with HIV-1 gp120 (Fig. 1A, lane 2), suggesting a possible dual infection, which is further examined below.

HIV-1 subtyping by HMA. HMA of HIV-1 *env* genes was first described by Delwart et al. (10) and has since been adapted for use in the rapid classification of HIV-1 strains into sequence subtypes (3, 4, 9). Past studies determined that heteroduplexes formed between members of the same sequence subtype have faster electrophoretic mobilities than heteroduplexes formed between members of different sequence subtypes, thus forming the basis for this analysis (3, 9). Here, 1.2-kb (V1 to V5) and 0.7-kb (V3 to V5) PCR products from the *env* gene were used to determine the genetic subtypes of the viruses in two HIV-1-seropositive individuals. For each, the 0.7-kb fragment provided the clearest subtyping result; an ethidium bromide-stained subtyping gel from one specimen is depicted (Fig. 2). The HIV-1 viral quasispecies of both samples were found to be heterogeneous (lane 1), suggesting that a diverse population of viruses exists within these individuals. Upon combining these uncharacterized HIV-1 strains and the reference sequences, an unequivocal determination of the sequence subtype for each showed that the heteroduplexes with the fastest mobilities, which migrated near the single strands, were those from subtype A (Fig. 2, lanes 2 to 4). Heteroduplexes from all other reference fragments migrated more slowly, between the single strands and the top of the gel (Fig. 2, lanes 5 to 16). Therefore, the HIV-1 sequences replicating within these individuals are of subtype A, the first reports of subtype A viruses in Sierra Leone. The viruses are designated HIV-1 93SL3 and HIV-1 93SL4.

HIV-2 *gag* and *env* analysis. The nested primer pairs designed for West Africa SIVsm (5), GF-GR1 for the first round and GF2A-GR3 for the second round, amplified *gag* fragments from both HIV-2 antibody-positive subjects (Fig. 3B). HIV-1-specific fragments were not amplified from genomic DNA of either HIV-2 specimen (Fig. 3A, lanes 1 and 2).

In addition, HIV-2 gene fragments were not amplified from the two HIV-1-infected individuals (Fig. 3B, lanes 3 and 4). Therefore, dual infection was not detected in any of the four HIV-positive subjects.

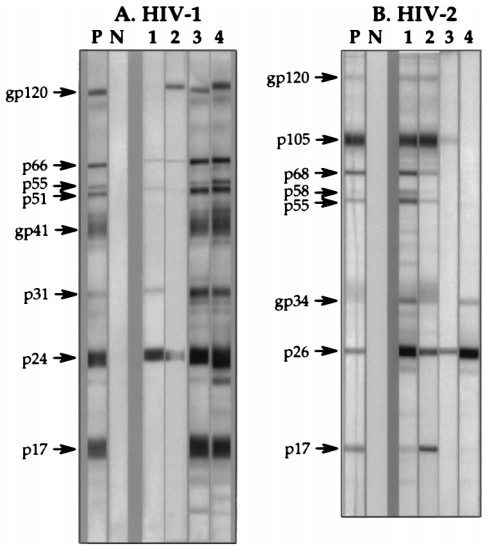

FIG. 1. Western blot analyses depicting the antigenic reactions of two HIV-2-positive sera, those with strains 93SL1 (lane 1) and 93SL2 (lane 2), and two HIV-1-positive specimens (lanes 3 and 4) to HIV-1 (A) and HIV-2 (B) proteins. P and N, positive and negative controls, respectively.

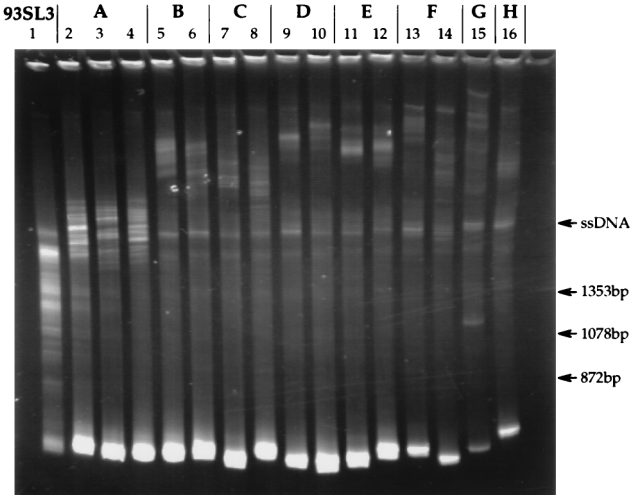

FIG. 2. HIV-1 subtyping by HMA. A PCR-amplified 0.7-kb *env* fragment (V3 to V5) from HIV-1 93SL3 was mixed and reannealed with standard HIV-1 subtypes (A to H) as indicated above the lanes. The faster mobilities of heteroduplexes whose reannealed strands showed highest homology allow HIV-1 93SL3 to be subtyped into clade A. The majority of bands migrating ahead of the single-stranded DNA (ssDNA) bands represent the heterogeneous viruses within the sample itself (93SL3 is in lane 1), whereas those bands near single-stranded DNA represent heteroduplexes with the reference sample (lanes 2 to 4). The locations of three molecular size markers are shown.

Transmembrane primers designed for SIVsm (5) failed to amplify transmembrane fragments from either HIV-2-seropositive subject, but newly modified primer pairs, EF4A-ER1A for the first round and EF5A-ER2A for the second round, succeeded in amplifying a 400-bp region of the transmembrane from one specimen only (data not shown). Because of the limited amount of genomic DNA, no other primers were tested. The two HIV-2 strains were designated HIV-2 93SL1 and HIV-2 93SL2 to indicate year of collection of specimen and country of origin. The last number designates the virus.

HIV-2 phylogenetic analysis. To define the evolutionary relationship of the newly identified HIV-2 viruses with other viruses within the HIV-2–SIVsm group, a 740-bp *gag* fragment and a 393-bp *env* fragment were PCR amplified, cloned, sequenced, and analyzed phylogenetically as reported previously (5, 6). PCR successfully amplified the *gag* fragments from both HIV-2 93SL2 and HIV-2 93SL1, but *env* was successfully amplified for HIV-2 93SL2 only.

The newly found HIV-2 strains are clustered within the SIVsm–HIV-2 lineage as previously described (5, 11). Amino acid sequence alignments of HIV-2 strains with *gag* (Fig. 4A) and *env* (Fig. 4B) are shown. To define relationships within the SIVsm–HIV-2 lineage, *gag* and *env* trees based on nucleotide sequences of previously described members of the SIVsm–HIV-2 lineage were constructed by neighbor-joining and bootstrap analyses (Fig. 5). One of the new HIV-2 strains, HIV-2 93SL1, clustered within subtype A. Its closest relative in the *gag* tree is HIV-2ST, which was previously identified in Senegal. HIV-2 93SL1 is the first HIV-2 subtype A to be identified and characterized from Sierra Leone. The subject from whom strain 93SL1 was found, who was infected with HIV-2 subtype A, was a physically healthy female itinerant trader, 40 years old, who acknowledged multiple sexual contacts in villages in the northern province of Sierra Leone. The fact that subtype A is now found in both Sierra Leone and Senegal, which do not border each other, establishes HIV-2 subtype A as no longer being restricted to contiguous countries in West Africa, as reported earlier (11). The most striking finding concerned HIV-2 93SL2, which did not cluster with any known HIV-2 subtypes in either the *gag* or *env* tree (Fig. 5). Because HIV-2 93SL2 was genetically equidistant from the five other HIV-2 subtypes, A to E, it therefore represents an entirely new sequence subtype, herein designated subtype F. The subject from whom strain 93SL2 was found was a 52-year-old female farmer who was also physically healthy. This subject acknowledged having had multiple sexual partners after her husband died several years ago as well as having consumed SM meat. The single occurrence of HIV-2 subtype F in 9,309 subjects in Sierra Leone and not elsewhere in Africa suggests that HIV-2 93SL2 is an example of an independent cross-species event of SIVsm into the human population of Sierra Leone.

Amino acid diversity of HIV-2 *env* and *gag* genes of primate lentiviruses of the SIVsm–HIV-2 lineage. Genetic diversity based on deduced amino acid sequences was calculated with Kimura's protein pairwise comparison formula (17). Table 2 shows the amino acid distances of HIV-2 93SL1 and HIV-2 93SL2 compared to those of other HIV-2 subtypes. For *gag*, the amino acid distance of HIV-2 93SL2 was greater than the intersubtype distances of four of five previously characterized HIV-2 subtypes (A to D). Based on *env* sequences, HIV-2 93SL2 is the most divergent of known HIV-2 subtypes. The *env* sequence from HIV-2 subtype E was not available for analysis. In contrast, HIV-2 93SL1 was not as divergent, based on a comparison of *gag* sequences, and was included with subtype A viruses. With the addition these two new HIV-2s found in

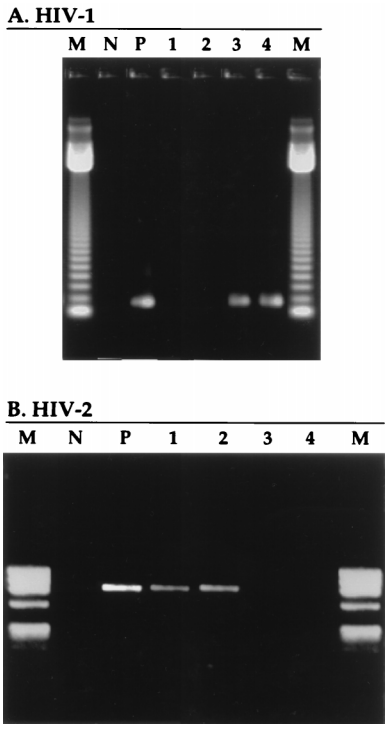

FIG. 3. Amplification by nested PCR of DNA from two HIV-1 (lanes 3 and 4)- and two HIV-2 (lanes 1 and 2)-seropositive subjects. (A) Lanes 3 and 4 contain HIV-1 0.2-kb *gag* fragments amplified from two subjects that were strongly seropositive for HIV-1. No product was obtained from subjects strongly seropositive for HIV-2. (B) Lanes 1 and 2 contain a 0.9-kb HIV-2 *gag* fragment from the two HIV-2-seropositive subjects. No bands were detected in the two HIV-1-infected plasmas. M, molecular weight markers; P and N, positive and negative controls, respectively.

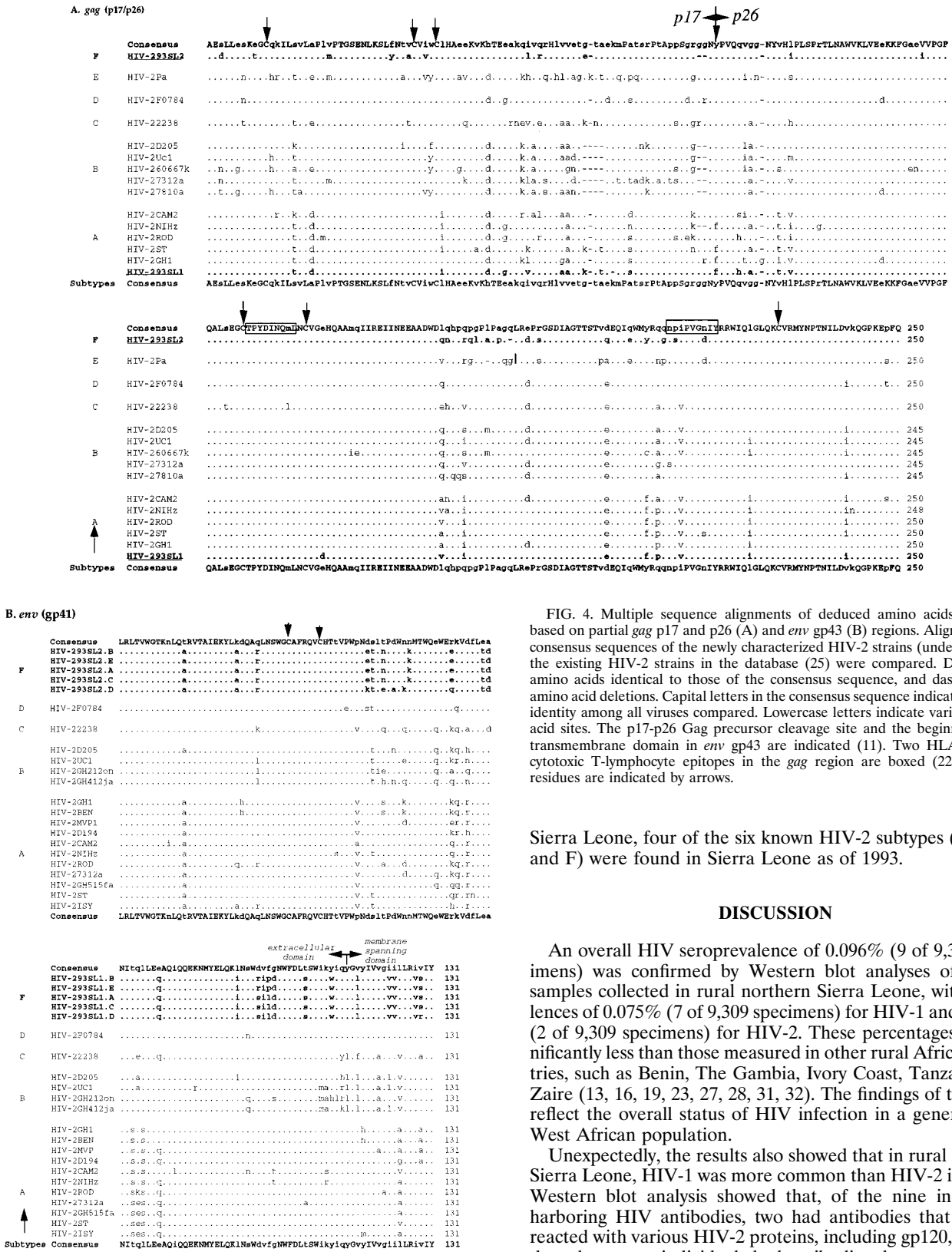

FIG. 4. Multiple sequence alignments of deduced amino acids of HIV-2 based on partial *gag* p17 and p26 (A) and *env* gp43 (B) regions. Alignments and consensus sequences of the newly characterized HIV-2 strains (underlined) and the existing HIV-2 strains in the database (25) were compared. Dots denote amino acids identical to those of the consensus sequence, and dashes denote amino acid deletions. Capital letters in the consensus sequence indicate sequence identity among all viruses compared. Lowercase letters indicate variable amino acid sites. The p17-p26 Gag precursor cleavage site and the beginning of the transmembrane domain in *env* gp43 are indicated (11). Two HLA-restricted cytotoxic T-lymphocyte epitopes in the *gag* region are boxed (22). Cysteine residues are indicated by arrows.

Sierra Leone, four of the six known HIV-2 subtypes (A, B, E, and F) were found in Sierra Leone as of 1993.

DISCUSSION

An overall HIV seroprevalence of 0.096% (9 of 9,309 specimens) was confirmed by Western blot analyses of plasma samples collected in rural northern Sierra Leone, with prevalences of 0.075% (7 of 9,309 specimens) for HIV-1 and 0.021% (2 of 9,309 specimens) for HIV-2. These percentages are significantly less than those measured in other rural African countries, such as Benin, The Gambia, Ivory Coast, Tanzania, and Zaire (13, 16, 19, 23, 27, 28, 31, 32). The findings of this study reflect the overall status of HIV infection in a general, rural West African population.

Unexpectedly, the results also showed that in rural northern Sierra Leone, HIV-1 was more common than HIV-2 infection. Western blot analysis showed that, of the nine individuals harboring HIV antibodies, two had antibodies that strongly reacted with various HIV-2 proteins, including gp120, whereas the other seven individuals had antibodies that reacted more strongly with HIV-1 antigens. Because these individuals had some antibody cross-reactivity between HIV-1 and HIV-2, specific PCR tests were further used to detect dual infections. However, of the four subjects successfully resampled and

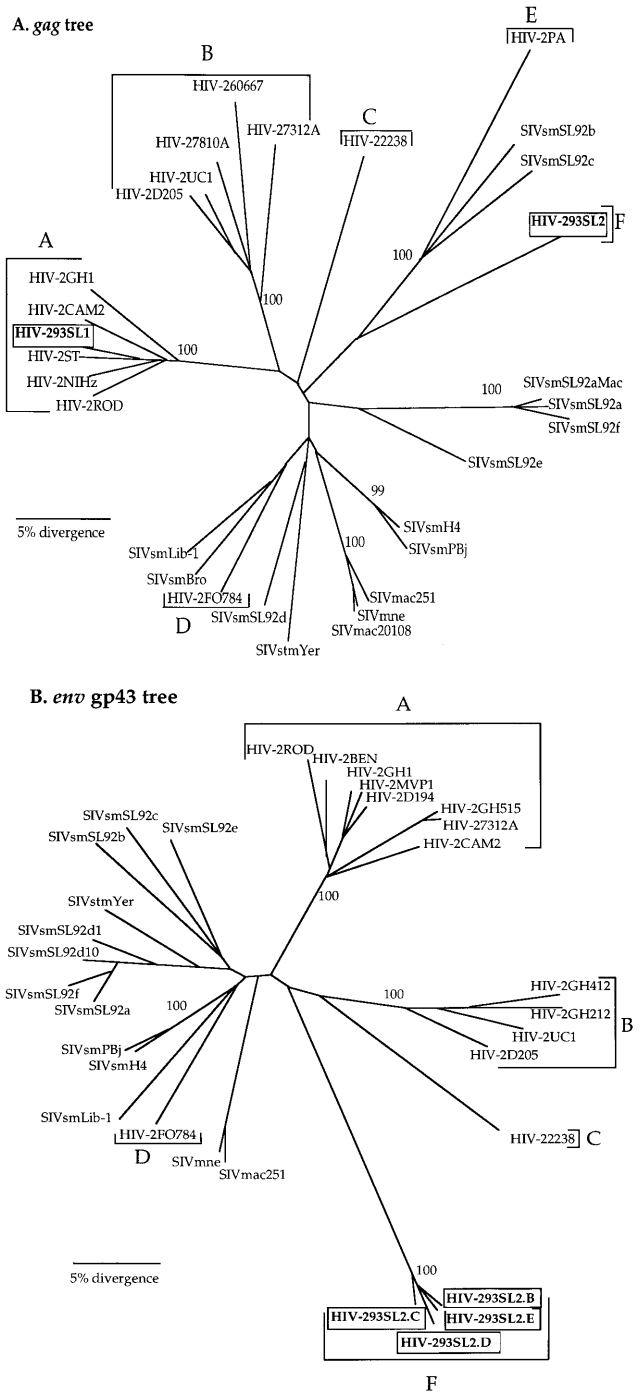

FIG. 5. Phylogenetic relationship between two newly characterized HIV-2 strains (boxed) and existing primate lentiviruses (unboxed) within the HIV-2–SIVsm lineage. Shown are trees based on *gag* sequences (A) and *env* gp43 sequences (B). Both trees were unrooted and constructed by the neighbor-joining method with nucleotide distance datum sets calculated by Kimura's two-parameter approach (17). No sequence gaps were included for analysis. In the *gag* tree, all six HIV-2 subtypes are indicated as A, B, C, D, E, and F (within brackets); in the *env* gp43 tree, subtype E was not available (11). The branch length is additive and proportional to the genetic distances between virus pairs. Values on the branches represent the percentages of 1,000 bootstrap replicates. Those bootstrap values not shown were less than 50%.

tested, no proviral HIV DNA suggestive of a dual infection was detected.

HIV-1 in rural Sierra Leone. Of the four individuals from whom follow-up samples were obtained, two of two with primarily HIV-1 antibodies were infected with HIV-1 (Fig. 1 to 3). Both of the new HIV-1 strains, HIV-1 93SL3 and HIV-1 93SL4, were subtype A, a finding which is not surprising because it is the most common subtype within Africa (19). However, this result is of interest for vaccine development and for tracking the spread of HIV-1 in West Africa.

HIV-2 in rural Sierra Leone. Phylogenetic analysis revealed that HIV-2 93SL1 is a new member of HIV-2 subtype A (Fig. 5). Indeed, it is the first subtype A HIV-2 strain to have been found in Sierra Leone. Most HIV-2 infections are caused by subtype A (11, 19), and its existence in this country provides more evidence for its widespread distribution in West Africa. A more striking finding, however, was that HIV-2 93SL2 is the most divergent strain based on its *env* sequence (Table 2) and, after subtype E, the second-most divergent HIV-2 strain based on its *gag* sequence. Moreover, both subtypes E and F have thus far been found only in Sierra Leone, suggesting that these viruses represent isolated occurrences of cross-species transmission to humans. Because HIV-2 93SL2 and HIV-2 PA are separated by two branching orders, these two HIV-2 subtypes most likely represent two independent crossovers of SIVsm into the human population.

The work described here provides further support for the hypothesis that the different HIV-2 subtypes were derived from different transmissions of SIVsm from SMs to humans. The fact that most individuals surveyed claimed to have had SM contact is plausible, considering SMs are frequently kept as household pets or hunted for food (21). As the prevalence of HIV-2 was much lower than that of SIVsm-infected feral SMs in the same country (28%) (5), HIV-2 is not transmitted as widely to West Africans as SIVsm is in its apparent natural host. It is not known how the subjects with strains 93SL1 and 93SL2 acquired HIV-2; although both had had contact with SMs as food items, the virus may have been sexually transmitted to both women. Regardless of the origin, the finding of the two most divergent subtypes of HIV-2, subtypes E (11) and F, only in persons from Sierra Leone may be highly significant because this is the same region of the world that contains divergent variants of SIVsm belonging to the HIV-2–SIVsm family (5). With the addition of subtypes A and F, four of the six known HIV-2 subtypes occur in Sierra Leone (Table 3). Moreover, one or more examples of all known HIV-2 subtypes occur in persons from Sierra Leone, Liberia, or Ivory Coast

TABLE 2. Amino acid distances from consensus sequences of *gag* and *env* gp41 genes of HIV-2 subtypes

Subtype	Amino acid distance from consensus of the indicated protein in the indicated subtype[a]										
	HIV-2 Gag						HIV-2 Env gp41				
	A	B	C	D	E	F	A	B	C	D	F
A	7.7						12.6				
B	13.9	10.9					26.2	12.3			
C	14.4	15.0	NA[b]				22.5	23.3	NA		
D	12.4	13.5	15.2	NA			17.7	22.0	25.7	NA	
E	24.1	21.7	24.3	21.5	NA		ND[c]	ND	ND	ND	
F	18.3	19.8	19.6	14.9	20.1	NA	29.5	31.0	34.6	24.9	NA

[a] Where a value is given, the intrasubtype distance is known.
[b] NA, not applicable, because only one viral strain was identified.
[c] ND, no data, *env* sequence for subtype E not available.

TABLE 3. Geographic distribution of HIV-2 subtypes in West Africa

HIV-2 subtype(s)	Viral strain(s)[a]	Geographic location[b]
A	93SL1	*Sierra Leone*
	7810A	*Ivory Coast*
	ST, MVP, and 60415K	Senegal
	ROD	Cape Verde Islands
	BEN	Mali
	GH1 and FA	Ghana
	ISY and D194	The Gambia
	NIHz, CAM2, and GB1222	Guinea Bissau
B	D205, GH2, ON, and FT	Ghana
	UC1 and 7924A	*Ivory Coast*
	JA	Nigeria
	60667K	*Sierra Leone*
A and B	7213A[c]	*Sierra Leone*
C	2238	*Liberia*
D	FO784	*Liberia*
E	PA	*Sierra Leone*
F	93SL2	*Sierra Leone*

[a] Newly identified HIV-2 strains are 93SL1 and 93SL2. Data on previously characterized HIV-2 strains were obtained from GenBank (25).

[b] The countries that are within the natural habitat of SMs (*Cercocebus torquatus atys*) are in italic type (33).

[c] 7213A is a recombinant strain between the A and B subtypes.

(Table 3). These are the only three countries on the African continent with SMs, which fact further points to SMs as the only source of HIV-2. Of African countries outside the range of the SM, Ghana has two HIV-2 subtypes and the rest have only one subtype (Table 3). The parallel distribution of divergent SIVsm and divergent HIV-2 viruses in the same small area of Africa (5, 12) provides further evidence for the scattered, sporadic crossover of divergent SIVsm strains to human beings in West Africa. How this transmission may have occurred remains to be determined.

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