Genetic Diversity of Simian Immunodeficiency Viruses from West African Green Monkeys: Evidence of Multiple Genotypes within Populations from the Same Geographical Locale

FRÉDÉRIC BIBOLLET-RUCHE,1* CÉCILE BRENGUES,1 ANH GATL-LUONG,2 GÉRARD GALAT,2 XAVIER POURRUT,2 NICOLE VIDAL,1 FRANCISCO YEAS,2 JEAN-PAUL DURAND,3† AND GÉRARD CUNY1

Laboratoire Rétrovirus, ORSTOM, B.P. 5045, 34032 Montpellier cedex 1, France, and Laboratoire de Primatologie, ORSTOM, BP 1386,5 and Laboratoire des Virus, Institut Pasteur, BP 220,3 Dakar, Senegal

Received 16 October 1995/Accepted 10 October 1996

High simian immunodeficiency virus (SIV) seroprevalence rates have been reported in the different green monkey (AGM) subspecies. Genetic diversity of these viruses far exceeds the diversity observed in the other lentivirus-infected human and nonhuman primates and is thought to reflect ancient introduction of SIV in the AGM population. We investigate here genetic diversity of SIVagm in wild-living AGM populations from the same geographical locale (i.e., sympatric population) in Senegal. For 11 new strains, we PCR amplified and sequenced two regions of the genome spanning the first tat exon and part of the transmembrane glycoprotein. Phylogenetic analysis of these sequences shows that viruses found in sympatric populations cluster into distinct lineages, with at least two distinct genotypes in each troop. These data strongly suggest an ancient introduction of these divergent viruses in the AGM population.

* Corresponding author. Mailing address: ORSTOM, Laboratoire Rétrovirus, 911, Ave. Agropolis, BP 5045, 34032 Montpellier cedex 1, France. Phone: (33) 67 61 74 64. E-mail: bibollet@infobiogen.fr.
† Present address: DIMIT, PHARO IUTSSA, 13007 Marseille, France.

Genetic diversity of lentiviruses has been extensively studied during the last decade. Based on nucleotidic and amino acid sequences of several genes, human immunodeficiency virus type 1 (HIV-1) isolates fall into two major genetic clusters, M and O (33, 46). This latter group was only recently described and is represented by a limited number of viruses of Cameroonian origin (7, 18, 26, 40, 61). Group M comprises the vast majority of HIV-1 strains all over the world and is further subdivided into nine distinct subtypes A to I, based on equidistant env and gag sequences (34, 41, 46). Separation of HIV-1 strains in two distinct clusters is thought to reflect multiple introductions of nonhuman primate viruses into the human population (58). Identification of simian immunodeficiency viruses (SIVs) in chimpanzees (Pan troglodytes) from Central Africa (50, 51) related to HIV-1, i.e., with the same genetic organization (25), is an argument in favor of this hypothesis. Similarly, HIV-2 strains have been divided into five distinct subtypes A to E, based on env and gag sequences. Analysis of the phylogenetic relationships between SIVsm/SIVmac (isolated from sooty mangabeys, Cercocebus aterrimus, and several species of captive macaques [5, 10, 48], respectively) and HIV-2 shows that some human strains strongly cluster with simian strains (24, 43). Here again, these results suggest multiple cross-species transmission from mangabeys to the human population (16).

Among lentiviruses, nonpathogenic SIVs from African green monkeys (Cercopithecus aethiops) (AGMs) form an independent lineage, equidistantly related to the other four phylogenetic groups used to classify primate lentiviruses (58).

High seroprevalence rates, from 30 to 50% (1, 19, 49), have been reported in these monkeys in the wild; thus they represent a large reservoir (1, 2, 14, 19, 35) and probably an important source of infection for other nonhuman primates in their natural habitat. Infection of heterologous species by SIVagm-related strains has been reported by several groups (6, 27, 60). Based on geographical distribution and phenotypic and genetic characteristics, AGMs have been classified into four subspecies: gri (Cercopithecus aethiops aethiops), vers (Cercopithecus aethiops pygerythrus), tantalus (Cercopithecus aethiops tantalus), and sabaeus (Cercopithecus aethiops sabaeus) (37, 56). Interestingly, each subspecies is infected by species-specific virus strains, referred to as SIVagm- gri, SIVagm-ver, SIVagm-tan, and SIVagm-sab, respectively. Differences between viruses infecting one AGM subspecies are always less than interspecies variations, and phylogenetic analyses for env nucleotidic or protein sequences have confirmed these observations (1, 3, 22, 29, 38, 39, 45). Together, these findings suggest that the four subspecies-specific SIVagm strains have evolved coincidently with their natural hosts and have diverged from a common ancestor.

To date, genetic diversity of these viruses has been studied by comparing independent isolates in populations of different geographic origins. The objective of this study was to determine genetic diversity of SIVagm-sab within AGMs of the same geographical locale. For this purpose, we PCR amplified and sequenced tat, env, and pol fragments from sabaeus viruses recovered from monkeys living in nonoverlapping home ranges, i.e., sympatric populations. Our data indicate the presence of distinct virus lineages, supporting a complex epidemiological pattern of SIVagm in naturally infected AGM populations.

MATERIALS AND METHODS

Animals and serology. The AGMs are from two social troops, living in nonoverlapping home ranges, i.e., sympatric populations, numbered P (P031, P032, P045, P051, P055, P056, P058, P081) and G (G021, G023, G024). Seroprevalence rates were comparable between the two groups: 19 of 40 (47%) and 8 of 17 (47%) for P- and G-numbered monkeys, respectively. Animals were trapped and sampled from September 1991 to January 1993 in the Fathala forest (Saloum Delta National Park, Senegal). Trapping and blood sampling have been described elsewhere (15). All animals were in good health and showed no clinical signs of an AIDS-like related disease at the time of sampling. We also included,
PCR amplification. Seminested PCR was used to characterize viral sequences from primary uncultured peripheral blood mononuclear cells (PBMC) (P031, P032, P045, G021, G023, and G024) and from chronically infected Molt4-cloned DNA (P031, P032, P055, P081, K033, and K042). Two reactions were amplified, 460 bp from the transmembrane glycoprotein (TMgp) and a 460-bp fragment containing the first 1 exon. Primers used are the following, and location corresponds to SIVagm-sab1c sequence (22): SV3 (outer forward), GTGAAACGTCACATTGG (8219 to 8242); PS6 (outer reverse), GAGCTCTTGCGACCATATCAT (8398 to 8315); EM1 (inner forward), GGATAATCAGGATACACTTTG (8478 to 8500) for env; TAT3 (outer forward), CAGGGGCGCTGAGATACCCCT (6081 to 6104); TAT2 (outer reverse), TAT3 (inner forward), CCAAGAATCTCTCCTTTCC (6688 to 6710). Genomic DNA was prepared with the Ready Amp genomic DNA purification kit from Promega, and 1 to 2 µg was used for amplifications. PCRs were performed in a final volume of 50 µl containing 5 µl of 10× PCR buffer [500 mM KCl, 100 mM Tris-HCl (pH 8.3), 50 mM MgCl2, 10 mM dithiothreitol, 0.5 mM spermidine, 1 µM each primer], 200 µM of each dNTP, 0.5 units of Taq DNA polymerase (Promega), and 0.5 µg genomic DNA. The thermal cycles used were as follows: 30 s at 94°C, 30 s at 55°C, and 72°C for 2 min, 25 cycles for the outer primers (SV3/PS6 for TMgp, TAT3/TAT2 for env region), 5 µl of the first amplification was subjected to 40 cycles as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final extension for 7 min.

RT-PCR. The pol region, 320 bp from the integrase gene, was amplified by reverse transcriptase PCR (RT-PCR) technique, with primers described by Minia et al. (46). Briefly, from 50 µl of plasma by the guanidium thiocyanate-phenol-chloroform method (8), was retrotranscribed at 1 h at 42°C with avian myeloblastosis virus (Promega) and reverse PCR oligonucleotide as primer, in a final volume of 20 µl containing 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM MgCl2, 10 mM diethanolamine, 0.5 mM spermidine, 1 µM each (inner forward), CCAAGAATCTCTCCTTTCC (6688 to 6710) and 0.5 µM primer each primer. Samples were overlaid with 100 µl of mineral oil to prevent evaporation and subjected to amplification in a Perkin-Elmer DNA thermal cycler. For each amplified region, homology conditions were 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min, 25 cycles for the outer primers (SV3/PS6 for TMgp, TAT3/TAT2 for env region), 5 µl of the first amplification was subjected to 40 cycles as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final extension for 7 min.

DNA sequencing. Amplified SIV pol, env, and tat genes were directly sequenced. PCR products were separated on SeaPlaque GTG (FMC BioProducts, Rockland, Maine) low-melting-point agarose gels, in 1× TAE buffer (Tris-HCl [90 mM], acetic acid [10 mM], EDTA [0.1 mM], pH 7.8) and 0.5 mg of ethidium bromide per ml, and visualized by short exposure to UV light. Bands were excised from the gel, and approximately 100 to 150 ng of DNA in 9 to 10 µg of agarose (assuming that 1 µg corresponds to 1 µl and 100% recovery) was used for sequencing reactions. Each fragment was sequenced in both strands with an Applied Biosystems sequencer (model 373A; Applied Biosystems, Inc.) and a dye-deoxy terminator protocol, as specified by the manufacturer. After cycle sequencing, samples were heated at 65°C for 10 min, to ensure melting of the reaction before phenol (water-saturated)-chloroform extraction and ethanol precipitation.

Sequence analysis. Overlapping sequences were joined by using SeqEd-1.0 (Applied Biosystem Inc.). Sequences were aligned by the CLUSTAL (20) program. Evolutionary distances were calculated using Kimura's two-parameter method with correction for the multiple substitutions and excluding positions with gaps in aligned sequences (31). Phylogenetic relationships were computed from the distance matrix by the neighboring method (57). Reliability of the branching orders was confirmed by the bootstrap approach (12). All these methods were implemented with CLUSTAL V (21).

Nucleotide sequence accession numbers. The sequences have been submitted to the GenBank database under accession numbers U37197 (env for G021, P055, P081, K033, and K042), U37199 (pol for K042, U37200 (K033), U37201 (pol for P055), U37202 (pol for P031), U37203 (pol for P055), U37204 (pol for P055), U37205 (pol for P055), U37206 (pol for P031), U37207 (env for K033), U37208 (env for K042), U37209 (env for P055), U37210 (env for P031), U37211 (env for P081), U37212 (env for P058), U37213 (env for P031), U37214 (env for G023), U37215 (env for G024), and U37216 (env for P031) and U57593 to U57602 for tat pol.

RESULTS

PCR and sequencing. To determine the genetic relationships among SIVs present in this population, we PCR amplified and sequenced env (460 bp from the TMgp) and tat (460 bp) regions for 11 new SIVagm-sab1b viruses found in naturally infected AGMs. In four cases, we failed to amplify the SIV fragment, despite repeated attempts under various amplification conditions: env for P051 and tat for P081, P045, and P032. The env fragment allowed assessment of the variability of 140 amino acid residues in the TMgp, corresponding to the transmembrane domain and a part of the cytoplasmic region. Determination of env and tat sequences allowed deduction of amino acid sequences for Tat and Rev regulatory proteins. Alignments of these deduced amino acid sequences and comparisons with other SIVagm strains are depicted in Fig. 1. For some of these viruses, a third region was characterized, 320 bp from the integrase gene.

Comparisons of TMgp sequences. For the part of the TMgp, an average identity of approximately 72% for protein sequences between the saeboes monkey viruses from sympatric populations is comparable to homology between SIVagm-verbatim viruses (Table 1). In addition, these values ranging from 58.6 (between K042 and G024) to 91.7% (between P031 and P035) in the saeboe group allow detection of, first, highly divergent strains (G024 and K042) and, second, closely related ones (P055, P056, P058, and P031). Globally, intergroup homology between SIVagm-sab and the other subspecies viruses is lower than the identity between vervets and grivets. Analysis of Tat sequences revealed conserved regions for G, P, and K viruses (Fig. 1), specific for SIVagm-sab viruses in the transmembrane domain and in the cytoplasmic domain. These regions show limited variability, with conservative amino acid changes (amino acids of the same class). A hypervariable domain is also observed at the same position as described for SIVagm-ver (3) and is extended towards the C terminus of the protein. In K042, an in-frame stop codon was found at the same position as observed for SIVagm-sab1c (28), G021 also contained an in-frame stop codon leading to a cytoplasmic tail of 70 residues, to be compared with a nontruncated form of approximately 150 amino acids found in P055 and P056 (data not shown).

Comparisons of Tat and Rev sequences. Analysis of Tat sequences revealed limited amino acid variability for the first coding exon and the domains known to be important for protein function (cysteine-rich, basic domain [Fig. 1]). A first interesting feature concerns variability in length and composition observed for the first 10 amino acid residues of the different Tat proteins (Fig. 1). This variability concerns insertion of basic (arginine and/or histidine) and proline residues preceding a highly conserved acidic sequence (QVWEELQEE). This part of the protein, by comparison with HIV-1 and HIV-2 Tat proteins, corresponds to the activation domain, and such variability has not been described previously in any other lentivirus for a regulatory protein. As observed for Tat, domains known to be responsible for Rev function in HIV-1 are conserved (basic and leucine domains). Detailed analysis of the Rev sequences, however, indicated that the C-terminal domain is poorly conserved between the different viruses.

Analysis of the 5' splice junction for the 5' splice site in the first 10 amino acids of the different Tat proteins (Fig. 1). This variability concerns insertion of basic (arginine and/or histidine) and proline residues preceding a highly conserved acidic sequence (QVWEELQEE). This part of the protein, by comparison with HIV-1 and HIV-2 Tat proteins, corresponds to the activation domain, and such variability has not been described previously in any other lentivirus for a regulatory protein. As observed for Tat, domains known to be responsible for Rev function in HIV-1 are conserved (basic and leucine domains). Detailed analysis of the Rev sequences, however, indicated that the C-terminal domain is poorly conserved between the different viruses.
HIV Sabaeus monkey PO31 82.9 74.2 79.9 91.7 87.5 90.4 71.9 73.7 80.2 64.4 73.6 70.9 40.9 43.8 42.3 36.6 protein, according to the splice site used. These allow the production of either a full-length or a truncated Tat

are found inverted at the same positions in GO21 and GO24, and consequently, a truncated protein will be produced. KO33 and KO42 encode an identical full-length Tat protein, 8 amino acid residues shorter at the C terminus compared with the full-length PO55 Tat.

downstream of TA/GT in PO55, PO56, and PO31, can allow the production of either a full-length or a truncated Tat protein, according to the splice site used. These two splice sites are found inverted at the same positions in GO21 and GO24, and consequently, a truncated protein will be produced. KO33 and KO42 encode an identical full-length Tat protein, 8 amino acid residues shorter at the C terminus compared with the full-length PO55 Tat.

![FIG. 1. Multiple alignment of deduced amino acid sequences for Rev, Env, and Tat proteins, compared with SIVSABIC. The amino acids for Env correspond to the transmembrane and cytoplasmic domains of the TMgp (start at amino acid 639 in SIVSABIC). For Rev and Tat, domains known to be important for protein function are underlined. For these sequences, the position of the two exons is indicated. In each alignment, dashes refer to gaps introduced to maximize alignment, dots refer to identical amino acids, and the symbol # represents stop codons.](image)

### TABLE 1. Amino acid sequence identity among SIVagm

<table>
<thead>
<tr>
<th>Monkey sp.</th>
<th>Isolate</th>
<th>P031</th>
<th>P032</th>
<th>P045</th>
<th>P055</th>
<th>P056</th>
<th>P058</th>
<th>P060</th>
<th>G021</th>
<th>G023</th>
<th>G024</th>
<th>SAB1</th>
<th>K042</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabaeus monkey</td>
<td></td>
<td>82.9</td>
<td>74.2</td>
<td>79.9</td>
<td>91.7</td>
<td>87.5</td>
<td>90.4</td>
<td>71.9</td>
<td>73.7</td>
<td>80.2</td>
<td>64.4</td>
<td>73.6</td>
<td>70.9</td>
</tr>
<tr>
<td>Vervet</td>
<td>TYO</td>
<td>82.9</td>
<td>74.2</td>
<td>79.9</td>
<td>91.7</td>
<td>87.5</td>
<td>90.4</td>
<td>71.9</td>
<td>73.7</td>
<td>80.2</td>
<td>64.4</td>
<td>73.6</td>
<td>70.9</td>
</tr>
<tr>
<td>SAB1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The sequences compared correspond to positions 639 to 796 in SIVSABIC (stop codon was omitted). Other SIVagm sequences were obtained from the Los Alamos HIV database (46). Percentages of identity were determined by pairwise alignment and comparison.*
Evolutionary relationships between these viruses. To determine the evolutionary relationships between these SIVagm-sab strains, phylogenetic analysis was performed for tat and env nucleotidic regions, by the neighbor-joining method. As described earlier, the viruses isolated from the sabaeus subspecies form a distinct phylogenetic group in trees constructed for both env and tat sequences (Fig. 3). Clustering of the different viruses shows that these sequences are organized in distinct groups and reveals the presence of distinct SIVagm-sab phylogenetic lineages within sympatric AGM populations. Branching order allowed the designing of at least three clusters, designated A, B, and C for the env and tat sequences. The A group contains four strains from P-numbered animals, and two strains from this same P troop cluster in a separate B group. Another group, C, contains divergent strains from the monkeys numbered with G. Thus, in P and G troops, at least two distantly related virus groups are found, which are also distantly related between the troops. The three groups, supported by high bootstrap values, contain most of the sequenced viruses. Some viruses were not designated as groups because either they are represented by only one sequence in each phylogenetic tree (G032, G051) or they represent a divergent virus in both trees (G023, sab1C). These strains may indicate the presence of other divergent viruses circulating in these AGM populations. Viruses found in AGMs from Casamance (K033 and K042), referred to as group D in the env and tat trees, can be considered another independent lineage. They are more distantly related to any SIVagm-sab viruses. When phylogenetic analysis was performed with Env amino acid sequences, an identical clustering pattern was observed (data not shown).

These results were further confirmed by the phylogenetic analysis of the pol region (Fig. 4), with a high conservation of the different groups. Phylogenetic results obtained with this pol region have been shown to be similar to those obtained with the entire pol gene sequence (44). One change was observed compared with results obtained for env and tat, concerning the position of the P056 isolate. In env and tat trees, it clusters with the A group and is distantly related to this group in the pol tree (see Discussion).

**DISCUSSION**

In this study, we investigated the genetic diversity of SIVagm-sab recovered from AGMs living in the same and
These two splice sites are used in vivo is not known, but for a full-length Tat protein containing the two exons. Whether example, both could be used at different stages of Vd replication codon generated after splicing between the two exons (TNA?). In some strains, P031, POS, P0.56, and P058, a second PutabVe not yet understood.

moved after culture in macaque PBMC or in experimentally virus propagation in cells of human origin, but that it is re-
premature stop codon in the TMgp occurred after prolonged
clone8, and GO21 was directly amplified from DNA prepared
SIVagm-sab viruses.

Interesting result concerns the premature stop codon for KO42
slices are also found in G021 and G024, but
only a truncated Tat protein can be encoded due to the pre-
sequence of a premature stop codon. In contrast, K033 and K042
produced only full-length Tat proteins by replacement of TAA
by CAA and in the absence of a second putative splice site. In
HIV-1, the second Tat exon has been shown to be dispensable for
transactivation through the TAR structure but required for
posttranscriptional activation of env gene expression (30). For
HIV-2, in which TAR structure is closer to the structure found
in SIVagm-sab, this second Tat exon has been shown to in-
crease binding affinity of the protein for the TAR RNA struc-
ture (53). Relevance of this feature in the SIVagm-sab model
could be investigated by comparing relative transcriptional ac-
tivation by these proteins through the SIVagm-sab long termi-
nal repeat TAR structure.

The N-terminal region of the Tat protein has been shown to
 correspond to an activation domain for HIV-1 proteins, and
deletion of amino acids preceding the cysteine-rich domain
completely abolishes the Tat activity (36, 52). This region
shows an important variability in length and composition in our
SIVagm-sab strains by insertion of basic and proline residues.
These additional amino acids could be of importance for Tat
activity and functions. We also observed an important variabil-
ity in the C-terminal region of Rev. In HIV-1, this domain has
been shown to be important for protein function (62, 64),
allowing multimerization of the protein (9) while interacting
with the Rev-responsive RNA element (42). Remarkably, such
variability has not yet been reported for lentivirus regulatory
proteins, and it could have important consequences for virus
biological characteristics in vivo.

Origins of the different SIVagm-sab lineages. Phylogenetic
analysis of three independent regions of the viral genome (pol,
tat, and env) allowed identification of distinct lineages of the
SIVagm-sab, equidistantly related, circulating in sympatric
populations. Some of these strains have been isolated by co-
culture, and others were directly amplified from PBMC of
seropositive monkeys. As shown by the phylogenetic analysis,
no selection was introduced by coculture, and these different
lineages reflect viral diversity in the wild. Failure to amplify tat
fragment from the B group, either from isolated virus (P081)
or directly from PBMC (PO45), suggests that they are geneti-
cally distant. This is also the case for the env region from P051.
However, these results suggest that these different viruses are
biologically equivalent for transmission and/or infectivity as
they are found simultaneously within sympatric populations.

As the region from which these monkeys came is geographi-
cally isolated, AGMs from which they were recovered could
not have had recent contacts with other populations. Genetic
follow-up of an SIVagm infection in its natural host has shown
a limited variability of viral sequences (4), even for the so-
called variable regions of the external envelope glycoprotein,
and cannot account for the observed diversity. Amino acid
identity reported for four SIVagm-tan isolates by Müller et al.
(45) was thought to reflect viral diversity within the whole
AGM subspecies, as these viruses were recovered from mon-
keys living in distant geographical areas. Our data indicate that
the same extent of diversity is found within sympatric popula-
ations. It is also important to note that our results, obtained by
phylogenetic analyses, are consistent with SIVagm subclassifi-
cation according to subspecies of origin. This is another argu-
ment in favor of ancient introduction of SIVagm in AGM
populations. One remaining question concerns the origins of
these highly divergent strains. Several hypotheses could be
proposed: either these viruses have diverged from a common
ancestor (star phylogeny) or this high diversity results from
multiple introduction of divergent viruses at different periods

**FIG. 4.** Phylogenetic tree obtained for pol sequences. Groups A, B, and C are the same as for env and tat trees, except for the position of the P036 isolate.
in the AGM population. In this latter case, one would expect to find SIVagm strains from independent AGM populations clustering together in the same phylogenetic group. This would imply that the different SIVagm-sab lineages could be found in the whole population and that they should have been spread during the speciation of the different AGM subspecies. Further characterization of SIVagm strains from various geographical locations is necessary to confirm this hypothesis.

**Actual evolution of these viruses.** Description of different subtypes in HIV-1 phylogeny has globally been coincident with the geographical origins of the isolates and has been thought to reflect recent introduction of HIV in human populations. For example, the A and D subtypes are prevalent in central and western Africa, and the B subtype is predominant in Europe and North America. All these subtypes have been shown to evolve rapidly with elevated mutation rates (for a review, see reference 58). In the case of the feline immunodeficiency virus, three env subtypes have been recognized but geographical boundaries are less clear, especially for the B subtype (59). Interestingly, viruses belonging to this group were recovered from animals with no evidence of disease, some even having low CD4 numbers. The authors conclude that this result suggests, firstly, an earlier entry of the viruses in the feline population compared with HIV-1 and, secondly, even if the virus is more adapted to its host than are human viruses, an ongoing evolution.

In the light of the results concerning the HIV-1 and feline immunodeficiency virus genetic diversity and evolution, the presence of multiple, divergent, and nonpathogenic SIVagm-sab strains within AGM populations confirms a very ancient SIV infection in AGMs. In the case of SIVagm from the sabaeus subspecies, analysis of a complete provirus sequence (28) has revealed that some regions of the genome are more closely related to the SIVsm/HIV-2 lineages, suggesting that recombination had occurred between divergent viral strains in different hosts. Such recombination events have also been reported for HIV-1 (54, 55) and HIV-2 (17), and it is highly probable that it has also occurred between SIVagm strains during their evolution. One remaining question is the actual importance of recombination between the different genotypes that we have found in our populations. Because of elevated seroprevalence rates in the wild, it is highly probable that AGMs could be infected by distinct SIVagm-sab strains, and recombination events could play an important role in generating viral strains with particular biological properties. Results obtained by comparison of phylogenetic trees for the three studied regions indicate that the P056 isolate could have a recombinant genome; in the pol tree, it is found in a separate group compared with env and tat trees. Further investigations should be performed to look for coinfection of monkeys by several genetically distinct viruses and to elucidate the role of recombination between these different strains in generating viruses with new biological properties. These studies could provide clues to a better understanding of evolution of human lentiviruses.

**ACKNOWLEDGMENTS**

We thank Martine Peeters and Eric Delaporte for helpful discussions and assistance in carefully reviewing the manuscript. We also thank Abdoulaye Traore and Mamadou Diara for help in the field and the Direction des Parcs Nationaux du Sénégal for permission to work in the Saloum Delta National Park. This work was supported by grants from the Institut de Recherche pour le Développement en Coopération (ORSTOM).

**REFERENCES**

24. Höirsch, V. M., R. A. Olmsted, M. Murphy-Corb, H. R. Purcell, and P. R.