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

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High simian immunodeficiency virus (SIV) seroprevalence rates have been reported in the different African 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.

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 (17), A to E, based on *env* and *gag* sequences. Analysis of the phylogenetic relationships between SIVsm/SIVmac (isolated from sooty mangabeys, *Cercocebus atys*, 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: grivets (*Cercopithecus aethiops aethiops*), vervets (*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,

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for comparison, two SIVagm-sab isolates (numbered with K) obtained from AGMs living in Casamance (Senegal). The distance of approximately 200 km between these two populations, i.e., allopatric populations, provides a reference for genetic diversity of SIVagm-sab between sympatric populations. All the AGMs used in this study had HIV/SIV-cross-reactive antibodies, as tested by commercial HIV-1 plus -2 enzyme-linked immunosorbent assay (ELAVIA-Mixt; Diagnostics Pasteur, Marnes la Cocquette, France), line immunoassay (INNO LIA HIV1+2; Innogenetics NV, Antwerp, Belgium), and commercial HIV-1 and HIV-2 Western blot (immunoblot) (New Lav Blot I and New Lav Blot II; Diagnostics Pasteur). Animals were considered seropositive when cross-reactive antibodies were observed against at least the two envelope glycoproteins of HIV-2. For some animals (P051, P055, P056, P058, P081, K033, and K042), virus isolation was performed as described previously (6).

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-clone8 DNA (P051, P055, P056, P058, P081, K033, and K042). Two regions were amplified, 460 bp from the transmembrane glycoprotein (TMgp) and a 460-bp fragment containing the first *tat* exon. Primers used are the following, and location corresponds to SIVagm-sab1C sequence (28): SV3 (outer forward), GTTGAAGCTGACCATTTGGGGTG (8219 to 8242); PS6 (outer reverse), GAGCTCTTGCCACCCATATTCAT (8938 to 8915); EM1 (inner forward), GGGAATATCAGTAACACATTGG (8478 to 8500) for *env*; TAT3 (outer forward), CAGGGAGCCGTGGGACGAATGGCT (6017 to 6041); TAT2 (outer reverse), CCATACTGGAATGCCATAAAACAC (6559 to 6535); TAT1 (inner forward), CCCAAGGAATCTCCTTTTCCG (6086 to 6107). 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 100 μl containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM (each) deoxynucleoside triphosphates, 2.5 U of *Taq* DNA polymerase (Promega), and 0.4 μM 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, thermocycling 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 *tat* region). For the second round (EM1/PS6 for TMgp, TAT1/TAT2 for *tat* 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 Miura et al. (44). Briefly, viral RNA, extracted from 50 μl of plasma by the guanidium thiocyanate-phenol-chloroform method (8), was retrotranscribed for 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 $MgCl_2$, 10 mM dithiothreitol, 0.5 mM spermidine, 1 mM (each) deoxynucleoside triphosphate, 0.5 μM reverse primer, and 5 U of avian myeloblastosis virus RT. Five microliters from the RT reaction was used for amplification, with the same conditions as described for the second round for *env* and *tat* regions.

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 [80 mM], sodium acetate trihydrate [66 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 mg of agarose (assuming that 1 mg 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 procedure, 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 Biosystems, Inc.). Sequences were aligned by the CLUSTAL (20) program. Evolutionary distances were calculated by 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 neighbor-joining 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 (*tat* G021), U37198 (*tat* G023), U37199 (*tat* K042), U37200 (*tat* K033), U37201 (*tat* P051), U37202 (*tat* G024), U37203 (*tat* P055), U37204 (*tat* P056), U37205 (*tat* P058), U37206 (*tat* P031), U37207 (*env* K033), U37208 (*env* K042), U37209 (*env* P081), U37210 (*env* P058), U37211 (*env* P032), U37212 (*env* P045), U37213 (*env* P031), U37214 (*env* G023), U37215 (*env* G024), and U37216 (*env* G023) and U57593 to 57602 for *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-sab 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. Combination of *tat* and *env* 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 sabaeus monkey viruses from sympatric populations is comparable to homology between SIVagm-ver viruses (Table 1). In addition, these values ranging from 58.6 (between K042 and G024) to 91.7% (between P031 and P055) in the sabaeus 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 TMgp 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 (QVWEELQEEL). 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 *tat* gene (Fig. 2) shows important differences compared with the SIVagm-sab1C splice site for *tat* (28). In this latter strain, presence of a putative TA/GT 5′ splice site will give rise to a truncated Tat protein (73 amino acids), restricted to the first coding exon. The same situation was found in G023 and P051. In contrast, the presence of another putative splice site, CA/GT, 6 bases

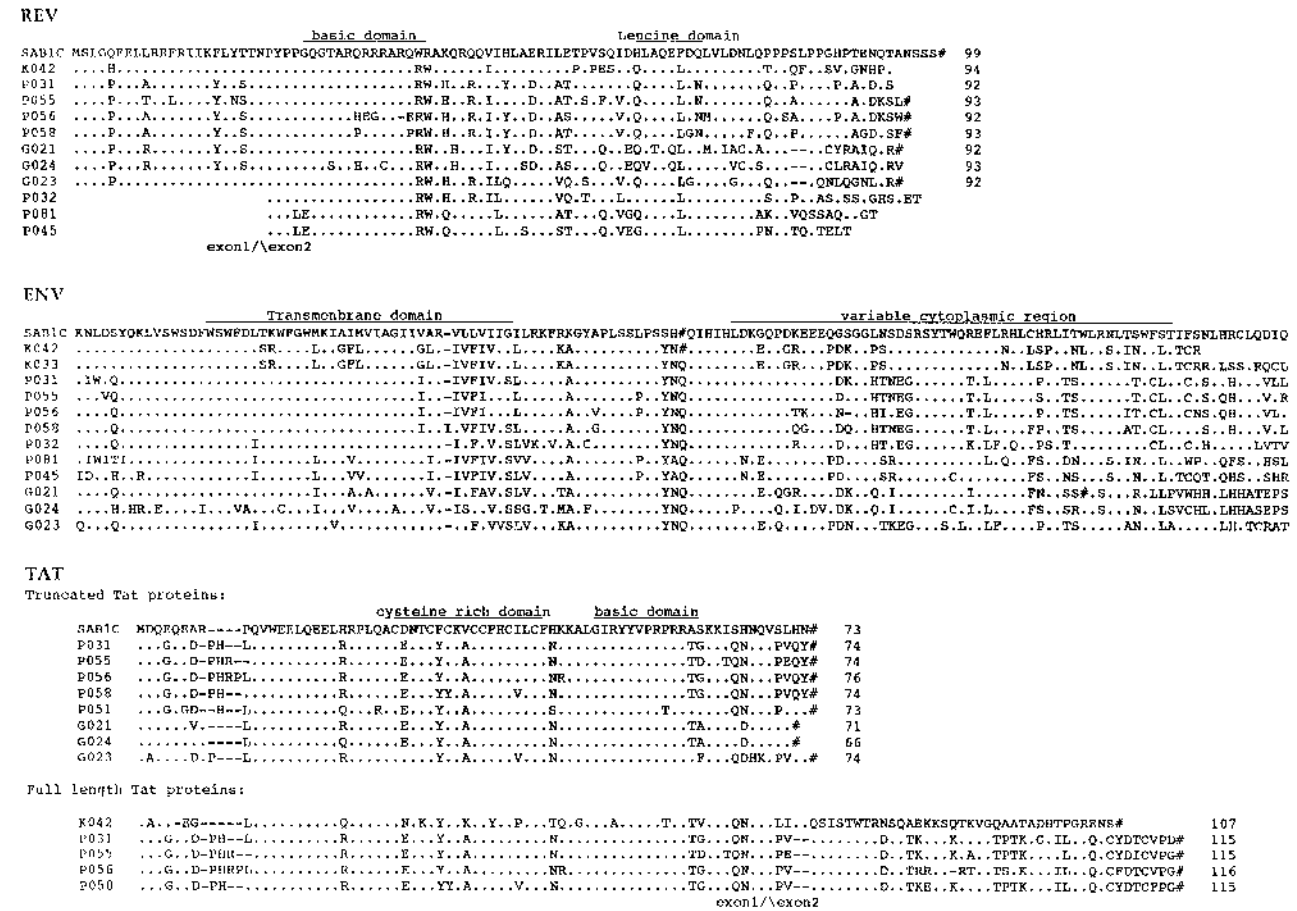

FIG. 1. Multiple alignment of deduced amino acid sequences for Rev, Env, and Tat proteins, compared with SIVSAB1C. The amino acids for Env correspond to the transmembrane and cytoplasmic domains of the TMgp (start at amino acid 659 in SIVSAB1C). For Rev and Tat, domains known to be important for protein 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.

downstream of TA/GT in P055, P056, P058, and P031, 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 G021 and G024, and consequently, a truncated protein will be produced. K033 and K042 encode an identical full-length Tat protein, 8 amino acid residues shorter at the C terminus compared with the full-length P055 Tat.

TABLE 1. Amino acid sequence identity among SIVagm[a]

Monkey sp.	Isolate	Sabaeus monkey											Vervet			Grivet 677
		P032	P045	P055	P056	P058	P081	G021	G023	G024	SAB1	K042	TYO	155	3	
Sabaeus monkey	P031	82.9	74.2	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
	P032		68.4	79.9	76.8	77.6	65.7	73.5	80.0	64.0	72.7	66.1	44.1	44.5	44.5	33.6
	P045			74.6	68.1	70.4	89.1	67.6	68.0	62.1	66.2	74.8	37.8	44.7	45.5	32.3
	P055				91.0	87.7	72.8	66.4	76.2	63.0	74.1	70.9	42.5	44.3	41.3	38.1
	P056					86.5	68.6	69.6	75.4	59.4	71.3	70.1	44.3	44.6	42.3	39.6
	P058						67.8	72.7	76.3	62.6	71.8	69.8	41.4	46.0	42.9	39.5
	P081							68.7	70.7	62.2	67.1	77.3	35.9	42.3	43.8	40.4
	G021								67.1	74.1	69.9	72.6	38.8	42.3	40.7	36.3
	G023									59.2	72.3	64.5	40.8	29.8	42.1	32.0
	G024										63.6	58.6	32.4	35.0	28.1	26.8
	SAB1											69.9	40.2	46.4	42.9	39.4
	K042												44.1	47.2	53.4	52.1
Vervet	TYO													66.9	70.0	51.0
	155														74.1	50.0
	3															50.9

[a] The sequences compared correspond to positions 659 to 796 in SIVSAB1C (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.

Strain	Sequence	Tat protein
SIVagm-SAB1C, G024, P051:	TACACAACTAGTAAGTATGA	
	TACACAACTA ATCCATATC (Δ)	→ truncated
P031, P055, P056, P058:	TACAGTACTAGTAAGTATGA	
	TACAGTACTA ATCCATATC (Δ)	→ truncated
or	TACA ATCCATATC (Δ)	→ full length
G021, G023:	TATAGTACCAGTAAGTATGA	
	TATAGTACCA ATCCATATC (Δ)	→ truncated
or	TATA ATCCATATC (Δ)	→ truncated
K033, K042:	TACACAACCAGTAAGTATGA	
	TACACAACCA ATCCATATC (Δ)	→ full length

FIG. 2. Putative splicing events for Tat proteins. In each case, the first sequence corresponds to the DNA sequence surrounding the splice sites, and the second represents juxtaposition of 5′ splice donor and 3′ splice acceptor sequences. Stop codons generated by splicing events are shown in boldface. The symbol Δ indicates a splice junction.

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 (P032, P051) 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

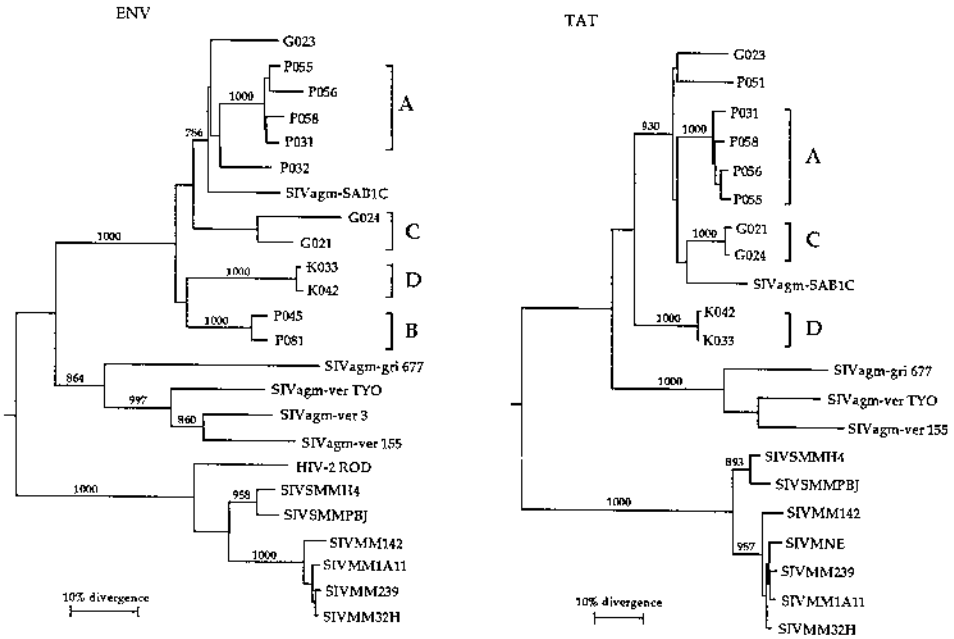

FIG. 3. Phylogenetic trees of SIVagm-sab subtypes derived from DNA sequences of partial *env* and *tat* genes. Phylogenetic relationships were determined by the neighbor-joining method as described in Materials and Methods, with SIV from mandrill as an outgroup (46). Clusters found in more than 750 of 1,000 bootstraps are indicated. Horizontal branch lengths are drawn to scale, while vertical branches are for clarity only. The different groups A, B, and C are indicated by brackets alongside the trees.

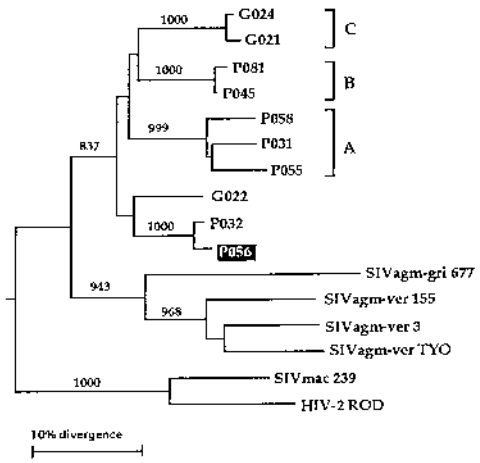

FIG. 4. Phylogenetic tree obtained for *pol* sequences. Groups A, B, and C are the same as for *env* and *tat* trees, except for the position of the P056 isolate.

distant geographical areas in Senegal (P- and G-numbered sympatric populations and K-numbered viruses). For 11 new strains, *env* and *tat* regions were amplified and sequenced. Results showed the presence of highly divergent viruses within these populations, with amino acid sequence differences ranging between 8 and 42% (mean, 28%). Even though limited data are available for this part of the Env protein, and also only a few SIVagm strains were characterized, these results indicate that genetic diversity of these viruses could be more important than previously described.

Comparison of sympatric and allopatric SIVagm-sab isolate envelope sequences. Alignments of Env amino acid sequences allowed detection of both conserved and variable regions, as well as short signature sequences of SIV from sabaeus subspecies. For the TMgp region that we have studied, identity was lower than previously reported for the C-terminal region of the external envelope glycoprotein (45), suggesting more divergence in TMgp between the different SIVagm groups. Another interesting result concerns the premature stop codon for K042 and G021 isolates. This feature has been reported for several lentiviruses. For SIVmac, it has been suggested that such a premature stop codon in the TMgp occurred after prolonged virus propagation in cells of human origin, but that it is removed after culture in macaque PBMC or in experimentally infected macaques (23, 32, 47). Such premature stop codons have also been reported for HIV-2 (63) and SIVagm (11) isolates, for which the origin of this premature stop codon is controversial (4, 13). Concerning our results on SIVagm-sab strains, K042 and K033 were isolated and propagated in Molt4-clone8, and G021 was directly amplified from DNA prepared from PBMC. Direct sequencing of the PCR products gave information about the major variant found in one animal. Thus, this premature stop codon in G021 is of biological relevance, and probably also in K042, for which its importance is not yet understood.

Particular features of regulatory proteins Tat and Rev from SIVagm-sab viruses. Most of the deduced amino acid sequences of Tat for P and G viruses contained a premature stop codon generated after splicing between the two exons (TA/A). In some strains, P031, P055, P056, and P058, a second putative alternative splice site 6 bp downstream allowed production of a full-length Tat protein containing the two exons. Whether these two splice sites are used in vivo is not known, but for example, both could be used at different stages of viral replication. Two splice sites are also found in G021 and G024, but only a truncated Tat protein can be encoded due to the presence 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 increase binding affinity of the protein for the TAR RNA structure (53). Relevance of this feature in the SIVagm-sab model could be investigated by comparing relative transcriptional activation by these proteins through the SIVagm-sab long terminal 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 variability 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 coculture, 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 (P045), suggests that they are genetically 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 geographically 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 monkeys living in distant geographical areas. Our data indicate that the same extent of diversity is found within sympatric populations. It is also important to note that our results, obtained by phylogenetic analyses, are consistent with SIVagm subclassification according to subspecies of origin. This is another argument 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

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.

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