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Simian Immunodeficiency Viruses from Central and Western Africa: Evidence for a New Species-Specific Lentivirus in Tantalus Monkeys

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Although up to 50% of African green monkeys (AGMs) are infected by simian immunodeficiency viruses (SIV) in their natural habitat, they remain asymptomatic carriers of these lentiviruses. They provide an attractive model to study not only the origin but also the link among genetic variation, host-virus adaptation, and pathogenicity of primate lentiviruses. SIVagm have been isolated from three species of AGM: the vervet (*Cercopithecus pygerythrus*), the grivet (*Cercopithecus aethiops*), and the sabaeus (*Cercopithecus sabaeus*) monkey. We studied four new SIVagm isolates from a fourth AGM species, the tantalus monkey (*Cercopithecus tantalus*), caught in the Central African Republic, and four new isolates from feral sabaeus monkeys from Senegal. Antigenic properties and partial *env* sequences were used to evaluate the diversity among these isolates. Alignment of *env* sequences in SIVagm isolated from tantalus and sabaeus monkeys permitted detailed mapping of the variable and conserved domains in the external glycoprotein. Genetic distances indicated that SIVagm isolates from tantalus monkeys are the most divergent among SIVagm in feral AGMs in Africa. The fact that AGMs are infected by four distinct lentiviruses, each specific for a single AGM species, supports the hypothesis of a coevolution of these viruses and their natural hosts and suggests that SIV transmission is a rare event among separated AGM species in the wild.

Currently, two human immunodeficiency viruses (HIV type 1 [HIV-1] and HIV-2) (7, 13) and six simian immunodeficiency viruses (SIV) are recognized. For HIVs, the nomenclature distinguishes the two different subtypes according to their differences in genetic organization and protein sequences (26). For SIVs, the present nomenclature indicates the species from which the virus was isolated. SIVmac was originally obtained from a captive rhesus macaque (*Macaca mulatta*) (14). Additional SIVs (SIVcyn, SIVmne, and SIVstm) were isolated from other macaque species in different U.S. primate centers (9, 23, 35). SIVs have also been isolated from naturally infected, nonhuman primates of African origin: SIVcpz from chimpanzees (31, 56), SIVsm from sooty mangabeys (21, 43, 48), SIVmnd from a mandrill (63, 64), SIVsyk from a Sykes' monkey (18), and SIVagm from African green monkeys (AGMs) (54).

According to sequence data, these human and simian lentiviruses had been classified into only four groups that do not follow the host species divergence (50). Indeed, humans can be infected by retroviruses belonging to two different groups, a unique situation in primate species. Conversely, HIV-2, SIVmac, and SIVsm belong to the same group although they have been isolated from different species. The situation is similar in the group containing HIV-1 and SIVcpz. Such a pattern of relationships between host species and viral genetic divergence has been explained by multiple hypothetical cross-species transmissions (29).

SIVs from AGMs were first considered to be only one group, characterized by an extremely high degree of genetic divergence (5, 19, 20, 34, 42). The significance of natural variability of SIVagm, however, was difficult to assess because the existence of different AGM species has only recently been considered (1).

AGMs form a superspecies according to their phenotypic differences (44) and geographic distribution (40). Four species are distinguished: vervets (*Cercopithecus pygerythrus*) are found from southern Ethiopia to South Africa, grivets (*Cercopithecus aethiops*) are limited to Ethiopia and the Sudan, tantalus monkeys (*Cercopithecus tantalus*) reside in the Central African Republic (C.A.R.), and sabaeus monkeys, commonly named west African green monkeys (*Cercopithecus sabaeus*), are restricted to west Africa (Fig. 1). SIVagm have been identified only in three of the four AGM species: in vervets, grivets, and sabaeus monkeys (1, 2, 16, 34, 37, 54), in which seroprevalence rates up to 50% have been demonstrated (27, 54). None of these seropositive AGMs showed clinical signs of immunodeficiency. The complete genomes of SIVagm isolates from three vervets and one grivet from east Africa have been sequenced (5, 19, 20, 34) and *pol* sequences of SIVagm from sabaeus monkeys have also been reported (2). However, SIVs from the fourth

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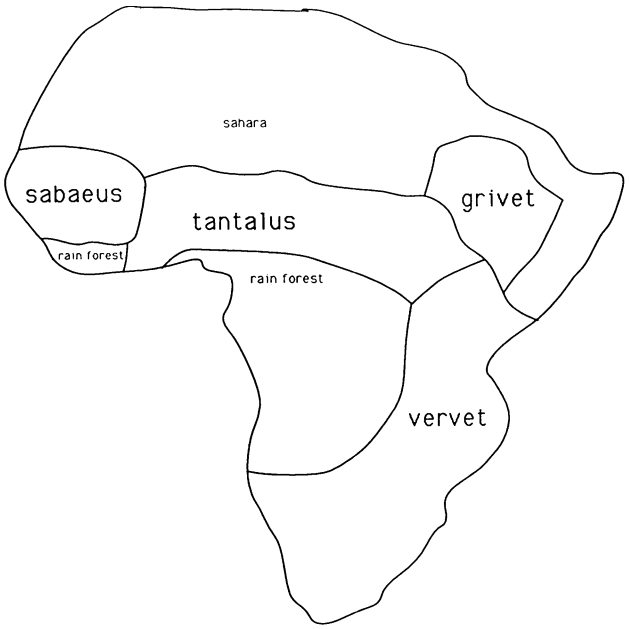

FIG. 1. Geographic distribution of the four AGM species: the grivet (*C. aethiops*), tantalus monkey (*C. tantalus*), vervet (*C. pygerythrus*), and sabaeus monkey (*C. sabaeus*) (55).

major AGM species, the tantalus monkey, have not been characterized. In addition, envelope variation maps, which provide information about structural constraints and immunological pressures, are still incomplete. We collected additional data about SIVs from AGMs, since this natural model of infection represents a key element in our understanding of the origin of primate lentiviruses and of the possible relationships among genetic variation, host-virus adaptation, and pathogenicity.

In the present article, we report the isolation and molecular characterization of SIVagm from four tantalus and four sabaeus monkeys, captured in the C.A.R. and Senegal, respectively. This allowed the evaluation of the genetic relationships among SIVs from all AGM species and a detailed mapping of variable and conserved regions in the external glycoprotein (EGP) of SIVagm from tantalus and sabaeus monkeys.

MATERIALS AND METHODS

Cells and viruses. Molt-4 (clone 8) (36) and SupT1 (62) T-cell lines were used to propagate SIVagm strains. SIVmac251 (14) was produced in the HUT78 cell line (24). All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and 1% L-glutamine.

Virus isolation. Eighty wild tantalus and 325 feral sabaeus monkeys were captured in several geographic regions of the C.A.R. and Senegal, respectively. These animals were all screened for their seropositive status by enzyme-linked immunosorbent assay (ELISA) (Elavia Ac-Ab-AkI, Elavia Ac-Ab-AkII; Diagnostics Pasteur) and Western blot (immunoblot) analysis (New LAV Blot I, New LAV Blot II; Diagnostics Pasteur). The monkeys selected for our study were housed in individual cages either in Bangui (C.A.R.) or in Dakar (Senegal) and given designations beginning with "B" or "D," respectively. The SIV strains described here were isolated from four seropositive tantalus monkeys from the C.A.R. (B05, B14, B30, and B53) and from four seropositive sabaeus monkeys from Senegal (D29, D30, D37, and D45). The simian viruses from the C.A.R. were isolated from peripheral blood mononuclear cells. After separation of blood cells by Ficoll Hypaque gradient centrifugation, monkey peripheral blood mononuclear cells were stimulated with staphylococcal enterotoxin A (Toxin Technology) at a concentration of 0.5 μg/ml during the first 3 days of culture. The cells were then cultured in the presence of interleukin 2 as previously reported (7). To maintain production of cytopathic SIV strains in peripheral blood mononuclear cells, lectin-stimulated human peripheral blood lymphocytes, enriched for $CD4^+$ cells by $CD8^+$ cell depletion by using immunoglobulin G-coated magnetic beads (Dynabeads; Dynal Inc.), were used for coculture. The presence of virus in each culture supernatant was assessed by measuring the reverse transcriptase activity as previously described (7).

Virus propagation. SIVagm isolates from the C.A.R. were propagated on SupT1 cells after coculture with human $CD4^+$ lymphocytes, while SIVagm strains from Senegal were grown on Molt-4 (clone 8) cells. The morphology of viral particles was analyzed by electron microscopy.

Radioimmunoprecipitation assay. Human lymphoblastoid T cells (5×10^6) were infected with single SIV isolates from either a tantalus monkey (B14) or a sabaeus monkey (D29). These cultures were incubated with 200 μCi of [^{35}S]cysteine per ml in cysteine-free medium for 12 to 18 h (47). Labeled virus present in culture fluids was concentrated by ultracentrifugation, lysed, and incubated with monkey sera. Sera used for radioimmunoprecipitation assays were obtained from three SIV-infected AGMs (B14, D30, and D45), from an SIVmac251-infected macaque, and from seronegative animals and inactivated (56°C, 30 min). Protein A-Sepharose-precipitated immune complexes were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by autoradiography.

PCR. One microgram of Hirt supernatant DNA (30) from SIV- or HIV-infected cell lines was analyzed by polymerase chain reaction (PCR) (59). The selected oligonucleotides corresponding to conserved sequences in *env* are shown in Table 1. Reactions were carried out with buffer containing 50 mM KCl, 10 mM Tris-hydrochloride (pH 8.3), 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate, 2.5 U of *Taq* DNA polymerase (Cetus Corporation), and 0.4 μM each primer. Amplification cycles were as follows: an initial denaturating step was carried out at 94°C for 3 min and was followed by 35 additional cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min (3 min during the last cycle). The specificity of amplified proviral DNA was analyzed by Southern blotting and hybridization with [^{32}P]dATP-labeled probes consisting of either an SIVmac142 recombinant clone (11) or a PCR-amplified and purified SIVagmB30 *env* fragment. Hybridization was performed with 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% SDS–5× Denhardt solution at 60°C, and membranes were exposed, after washing, to Amersham multipurpose Hyperfilms.

Sequencing of PCR products. SIV isolates from four tantalus (B05, B14, B30, and B53) and four sabaeus (D29, D30, D37, and D45) monkeys were selected for sequencing. *env* proviral DNA fragments were obtained by PCR with the primer pair NS3s-NS3as (Table 1), except for the SIVagm B05 fragment, which could be successfully amplified only with the primer couple AE4s-NS3as. In addition, proviral DNA from SIVagmB14 was amplified with primers AE1s-AE3as and AE4s-AE5as. Purification of PCR products (0.5 to 3 μg) was performed on low-melting-point agarose gels

TABLE 1. Oligonucleotides for PCR and direct sequencing

Primer	Location in SIVagm *env*[a]	Specificity	Sequence (5′–3′)
AE1s	5849–5868	SIVagm	TTTATGGAGTACCAGTATGG
AE3as	6532–6551	SIVagm	CAATGCACTACTGAAACATT
AE4s	6554–6574	SIVagm	CAGGCTTAATGAATACTACAG
NS3s	6699–6722	SIVagm, SIVmac, HIV-2	TGTAGGAGACCAGGAAACAAGACA
AE5as	6913–6931	SIVagm	CTGGGTCTCCCCATTGTCT
AE5s	6913–6931	SIVagm	AGACAATGGGGAGACCCAG
MMas3	7150–7169	SIVagm	CCTTCTCTTGGCGGAGCATA
NS3as	7380–7403	SIVagm, SIVmac, HIV-2	AAGCCTAAGAACCCTAGCACAA

[a] Locations were determined from the nucleotide sequence of SIVagm(tyo-1) (20).

(GTG NuSieve). The band corresponding to the specific proviral DNA fragment was excised and the sequencing reactions were carried out directly with melted agarose (38). Briefly, 10 μl of amplified denatured DNA was hybridized at 37°C for 20 min with 100 ng of one of the primers already used for PCR. Labeling and termination reactions were then performed at 37°C with a Sequenase kit (U.S. Biochemicals). Sequencing reactions on each DNA strand were repeated with nested primers (NS3s, AE5s, and MMas3; Table 1). To control for artifacts, each sequencing reaction was repeated with PCR products from several independent amplifications. Samples were concentrated before being loaded on 6% acrylamide gels. Gels were exposed to Kodak X-Omat AR films for 12 to 48 h.

Nucleotide sequence accession numbers. The sequences have been submitted to the GenBank data base (Los Alamos, N.M.) under accession numbers M80208 (B14), M81070 (B05), M81071 (B30), M81072 (B53), M81073 (D29), M81074 (D30), M81075 (D37), and M81076 (D45).

RESULTS

Isolation and propagation of SIVs from feral tantalus and sabaeus monkeys caught in central and western Africa. Thirty of 80 wild caught tantalus monkeys (37%) and 131 of 325 feral sabaeus monkeys (40.3%) were found to be seropositive (17, 28). SIV isolates were recovered from four seropositive green monkeys from the C.A.R. (B05, B14, B30, and B53) and from four seropositive animals from Senegal (D29, D30, D37, and D45). Viruses were propagated on SupT1 and Molt-4 (clone 8) cells, respectively. Electron microscopy revealed the presence of typical HIV- and SIV-like lentiviral particles in all cultures by 10 days after infection (data not shown).

Antigenic homologies and differences between SIVs from tantalus and sabaeus monkeys. The supernatants from labeled SIVagmB14- and SIVagmD29-infected cultures were tested by radioimmunoprecipitation for reactivity with sera from one seropositive tantalus (B14) and two seropositive sabaeus monkeys (D30 and D45). In concentrated material from each supernatant of the two infected cell lines, a specific high-molecular-weight protein, the EGP, was recognized by all three sera (Fig. 2A and B, lanes 2 to 4). No significant difference in the sizes of these glycoproteins was observed. Similar results were obtained with sera and viral isolates from other SIV-infected AGMs from the C.A.R. and Senegal (data not shown).

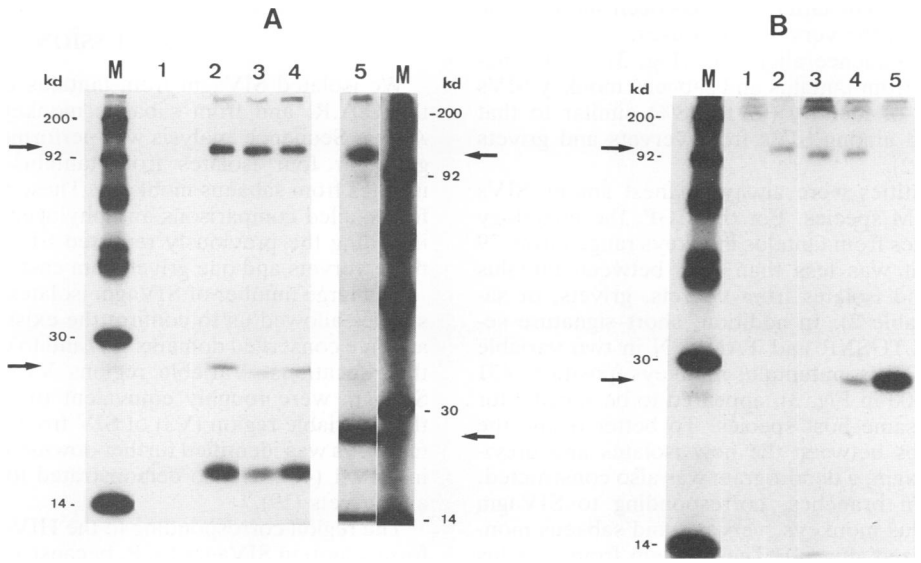

FIG. 2. Antigenic reactivities of SIV-seropositive sera against SIV strains from a tantalus monkey (B14) (A) and a sabaeus monkey (D29) (B) were analyzed by radioimmunoprecipitation assays. Labeled viral proteins were immunoprecipitated with serum samples from a seronegative monkey (lane 1); from seropositive AGMs B14 (lane 2), D30 (lane 3), and D45 (lane 4); or from a SIVmac251-infected macaque (lane 5). The arrows indicate the EGP and the major core protein. Molecular mass standards (M) were included in each gel.

The immune response of seropositive tantalus and sabaeus monkeys to SIV *gag* proteins was absent or low (Fig. 2A and B, lanes 2 to 4). This was also reported for SIV-infected vervets and grivets (1, 52). Why AGMs are apparently low responders to *gag* is still not clear.

Some antigenic differences were demonstrated between SIVagm from tantalus and sabaeus monkeys. Divergence was observed in the degree of cross-reactivity with the EGP of SIVmac: the putative gp120 of SIVagmD29 from a sabaeus monkey was not recognized by anti-SIVmac antibodies, in contrast to the EGP of SIVagmB14 from a tantalus monkey (Fig. 2A and B, lanes 5). In addition, SIVagmB14 and SIVagmD29 appeared to be different regarding the estimated size of their major *gag* products: the putative p26/27 of SIV from sabaeus monkeys appeared to migrate more slowly than that of tantalus SIVs (data not shown). This protein had also been reported to be larger than those of SIVs from vervets and grivets (2).

SIV isolates from tantalus monkeys are distinct from other SIVs and form a species-specific group. The study of the genetic relationships among SIVs from different AGM species was then focused on the *env* region that showed size variability of PCR products (data not shown). This region encodes the putative CD4 binding site (39) as well as three variable domains. PCR products of proviral DNA from four SIVs from tantalus monkeys (B05, B14, B30, and B53) and four SIVs from sabaeus monkeys (D29, D30, D37, and D45) were each sequenced. It should be noted that such PCR-derived sequences do not represent the entire virus population infecting a given animal. Direct sequencing of PCR products defines only the major genotype present in culture. However, viral sequences derived from the same individual are known to be very similar (6, 10, 41), and our data can be used to analyze divergence between SIVs from different AGM species. The deduced amino acid sequences of the external envelope proteins from the new SIV isolates were compared with the corresponding sequences of already reported SIVagm from Kenya and Ethiopia, namely SIVagm (tyo-1) (20), SIVagm155 (34), and SIVagm3 (5), which represent viruses from vervets, and SIVagm677 (19), which was isolated from a grivet. The latter virus has been shown to be highly divergent from the vervet lentiviruses.

The amino acid sequence alignment (Fig. 3) of the analyzed EGP regions from tantalus and sabaeus monkey SIVs indicated a degree of identity (57 to 68%) similar to that previously reported among SIVs from vervets and grivets (62 to 68%) (Table 2).

Amino acid identities were always highest among SIVs from the same AGM species. For the EGP, the homology between viral isolates from tantalus monkeys ranged from 79 to 90%, whereas it was less than 70% between tantalus monkey isolates and isolates from vervets, grivets, or sabaeus monkeys (Table 2). In addition, short signature sequences, such as LTGSXR and TANLEN in two variable EGP domains of SIVs from tantalus monkeys (positions 431 to 436 and 495 to 500 in Fig. 3), appeared to be specific for SIVagm from the same host species. To better define the genetic relationships between the new isolates and previously reported SIVagm, a dendrogram was also constructed. Four main SIVagm branches, corresponding to SIVagm from grivets, tantalus monkeys, vervets, and sabaeus monkeys, were recognized (Fig. 4). The SIVagm from tantalus monkeys branched before the other SIVagm. The SIVagm from sabaeus monkeys showed the least distance to SIVagm from vervets.

Mapping of variable and conserved *env* regions of SIVagm. The entire EGP coding region for one SIV isolate from a tantalus monkey (B14) has been sequenced (data not shown). The deduced amino acids were compared with the published EGP sequences of SIVs from three vervets and one grivet (Fig. 5) (5, 19, 20, 34). Five conserved and five variable regions could be distinguished. The fourth conserved region corresponds to the putative CD4 binding site (39) and was conserved in SIVagm. In addition, 18 cysteine residues were remarkably conserved between HIV-1 and SIVagm. However, five additional cysteines were present and conserved between all isolates of SIVagm. Four of them, which probably form two additional disulfide bridges in the second loop of the EGP, were also conserved in HIV-2 (50). The fifth cysteine was located in the fourth variable region (V4) of the EGP and may form, at least in some SIVagm strains, a disulfide bridge with a less conserved cysteine residue in the same region (Fig. 3).

The locations of the variable domains (indicated in Fig. 5) were similar to those defined for HIV-1, except for V3. The third variable region in SIVagm was found nearer to the C terminus than V3 in HIV-1. The domain whose location corresponds to the V3 loop in HIV-1 was conserved in SIVs from tantalus and sabaeus monkeys (Fig. 6). The hypervariable regions, showing more than 40% of variable sites, were V1, V2, and V4 (Fig. 5). In SIVagm, hypervariability was previously known for V1 and V2 (4) but not for V4, for which each site is variable except for the cysteines (Fig. 3). In addition, the EGP fragments of SIVs from the tantalus monkeys were characterized by a larger size due to two large insertions in the V4 region, which probably accounted for the differences in electrophoretic mobility of PCR products from SIVs of tantalus and sabaeus monkeys. Such differences may not have been reflected by SDS-PAGE after radioimmunoprecipitation of the viral proteins (Fig. 2), because this method does not permit precise definition of the size of large molecules. Also, insertions in V4 of SIVagm from tantalus monkeys may be counteracted by deletions elsewhere in the EGP or by differences in glycosylation density compared with the EGP of SIV from sabaeus monkeys.

DISCUSSION

We isolated SIVagm from tantalus monkeys captured in the C.A.R. and from sabaeus monkeys captured in west Africa. Sequence analysis was performed with the envelope gene for four isolates from tantalus monkeys and four isolates from sabaeus monkeys. These sequences were used for detailed comparisons and phylogenetic reconstructions, including the previously reported SIVagm sequences from three vervets and one grivet from east Africa (5, 19, 20, 34).

The large number of SIVagm isolates from different AGM species allowed us to confirm the existence of five variable and five conserved domains (19) and to define more precisely their locations. Variable regions V1, V2, V4, and V5 in SIVagm were roughly equivalent to those of HIV-1. The third variable region (V3) of SIV from tantalus and sabaeus monkeys was identified further downstream than the V3 loop in HIV-1 (46), as also demonstrated for SIVs from vervets and grivets (19).

The region corresponding to the HIV-1 V3 loop might also form a loop in SIVagm EGP, because of the conservation of the two cysteine residues that delineate the loop in HIV-1. However, given the high sequence conservation of this domain in all SIVagm, one could suspect that this region is not subject to immunological pressure in the host. Neutral-

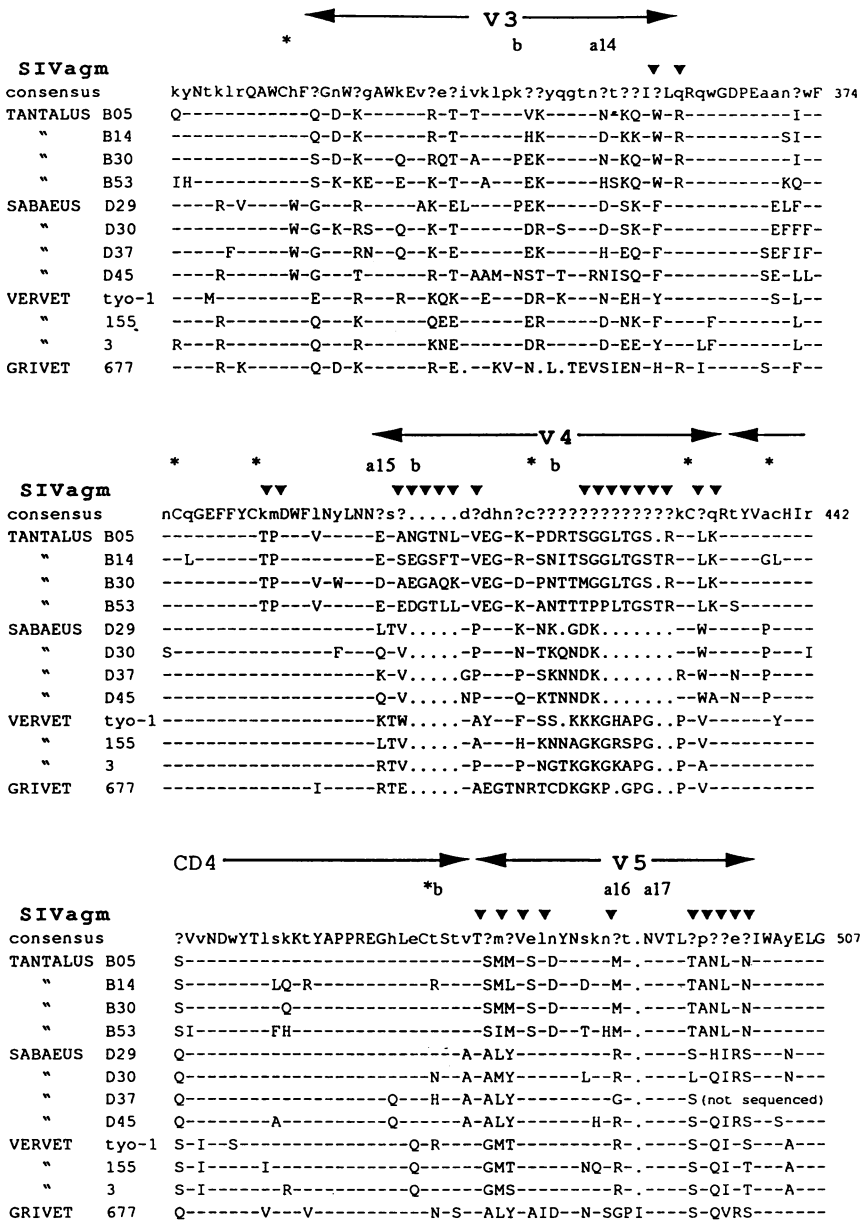

FIG. 3. Multiple alignment of deduced amino acid sequences of SIV isolates from four AGM species. The compared amino acids correspond to the C-terminal half of the EGP [amino acids 334 to 507 in SIVagm(tyo-1) (20)]. The putative CD4 binding site (34) is designated by analogy with HIV-1. V3, V4, and V5 designate variable regions. Triangles above the sequences indicate amino acids specific for tantalus monkey SIVs. Asterisks mark conserved cysteine residues. a14, a15, a16, and a17 indicate conserved potential glycosylation sites, and b indicates nonconserved potential glycosylation sites in SIV *env* from tantalus or sabaeus monkeys, according to the nomenclature proposed by Myers et al. (49). Capital letters in the consensus sequence represent conserved amino acids, and lowercase letters designate amino acids which are present in at least 50% of the sequences. Question marks indicate hypervariable sites. Dots in the sequences refer to deletions, and dashes refer to identical amino acids. Sequences from east African SIVagm were obtained from the Human Retroviruses and AIDS data base (49). Alignments were performed by using the Vizzspan computer program (60).

izing antibodies have been detected in SIV-infected AGMs, although to a lower level than in HIV-1-infected humans (52). It is not well known which epitopes are recognized by these antibodies. A recent study indicated that neutralizing determinants are absent in the HIV-1 V3 corresponding region in SIVagm (57). Similar to the observations reported for the EGP of HIV-2 and SIVmac (8, 32, 57), the principal neutralization determinant may rather lie within one of the hypervariable domains such as V1, V2, or V4. Indeed, the studied viruses appeared more closely related to HIV-2 than to HIV-1 when we compared the number and location of putative disulfide bridges in the EGP and regarding previously reported *env* sequence homologies between SIVagm and HIV-2 (25). But the HIV-1 V3 corresponding region in SIVagm could also contain such an epitope and escape, nonetheless, immunological surveillance by being only transiently exposed on the surface of the glycoprotein because of a conformational change occurring after binding to cellular

TABLE 2. Sequence homologies among SIVagm lentiviruses[a]

Monkey species	SIVagm	Sequence homology (%) among SIVagm from:										
		Tantalus monkeys			Sabaeus monkeys				Vervets			Grivet
		B14	B30	B53	D29	D30	D37	D45	TYO	155	3	677
Tantalus monkey	B05	85.6	90.1	82.9	68.0	65.9	57.1	64.7	67.2	69.1	68.6	63.2
	B14		84.0	78.6	66.3	65.9	65.2	60.6	65.5	68.0	65.7	60.8
	B30			81.2	66.9	65.3	66.5	63.5	66.7	67.4	66.9	60.2
	B53				63.3	62.4	63.9	59.4	60.9	62.9	62.3	57.9
Sabaeus monkey	D29					82.8	82.2	77.5	72.2	77.5	76.9	65.7
	D30						82.3	76.5	71.2	72.9	72.9	62.9
	D37							79.7	70.3	72.2	72.8	61.9
	D45								65.9	70.6	68.2	62.3
Vervet	TYO									78.2	79.9	61.8
	155										86.3	67.8
	3											66.1

[a] The numbers represent percent amino acid identities. The compared sequences correspond to positions 403 to 576 of the EGP in SIVagm(tyo-1) (20). SIVagm sequences of east African isolates were obtained from the Los Alamos HIV sequence data base (49). Percents were determined by a distance matrix method (51). Insertions and deletions were included in the calculation.

receptors (3). Thus, it is still not clear whether this conserved region in SIVagm contains a neutralizing epitope like that in HIV-1.

The EGP sequence showed a high degree of amino acid identity among SIVs from the same AGM species (up to 90% in tantalus monkeys). It should be pointed out that the new SIV isolates from tantalus monkeys were recovered from monkeys caught in different geographic areas of the C.A.R.: B05 and B14 in the central region; B30 in the south, near the border of Zaire; and B53 in the north of the country at the border with Chad. We therefore considered the observed homology to be representative of viral diversity within the tantalus monkey species, as it is not biased by selection of animals from the same colony. In contrast, the genetic distances between SIVs from different AGM species were significantly higher than intraspecies variability. Indeed, the minimum and maximum degrees of amino acid identity detected between the EGP sequences were 57.1% (tantalus and sabaeus monkeys) and 77.5% (sabaeus monkeys and vervets). Thus, viruses previously designated as SIVagm are clustered into different groups according to both criteria used for classification of human and nonhuman primate lentiviruses: species of their natural hosts and genetic divergence. These observations support the use of a different name for SIVs from each AGM species, i.e., SIVagm.ver, SIVagm.gri, SIVagm.sab, and SIVagm.tan for lentiviruses isolated from vervets, grivets, and sabaeus and tantalus monkeys, respectively (1).

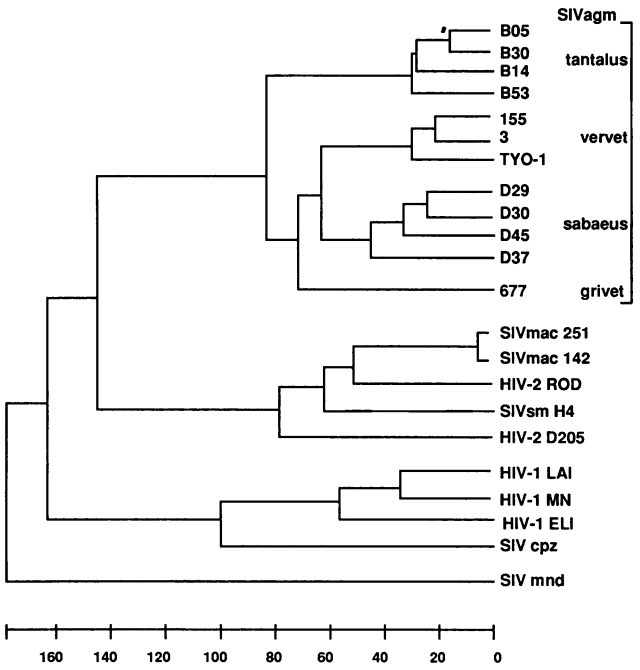

FIG. 4. Distance tree depicting the relationship in *env* of SIVagms from tantalus and sabaeus monkeys to other known SIVs and HIVs. The alignment was based on 582 nucleotides in *env*, deletions and insertions included [nucleotides 6769 to 7291 in SIVagm(tyo-1) (20)]. The sequences from tantalus and sabaeus monkey lentiviruses were derived from PCR products. The other sequences were obtained from the Los Alamos sequence data bank (49). The rooted tree is based on pairwise nucleotide divergence and is built by using the NJ clustering method (51). Horizontal distances are proportional to the average number of nonsilent substitutions. Similar tree topologies were obtained by using conserved *env* regions for the construction of dendrograms, by counting all mutations, and by excluding deletions and insertions.

The branching orders in the trees established for *env* and *pol* (2) of SIVagm or host proteins (58) appeared to be different. However, there may be ambiguity in the deeper branches of the green monkey phylogeny, which is based on only one electrophoretic study of 14 proteins and which does not include the tantalus monkey (58), and SIVagm trees, which are based only on *env* and *pol* gene fragments of 12 and 9 viral strains (2), respectively.

The existence of a distinct SIVagm for each of the four major AGM species supports the hypothesis of an infection of green monkeys before their speciation, as previously proposed (2). The absence of lentiviruses in AGMs brought to the Caribbean islands in the 17th and 18th centuries in conjunction with the slave trade (15) may argue against such an interpretation of SIVagm species specificity. However, vertical transmission has not been analyzed in AGMs and is below 40% in the case of HIV-1. In addition, the animals which accompanied the slaves were probably pets living with humans since their very young age or could have belonged to a seronegative colony of monkeys (15). Indeed, a detailed epidemiological survey of sabaeus monkeys in

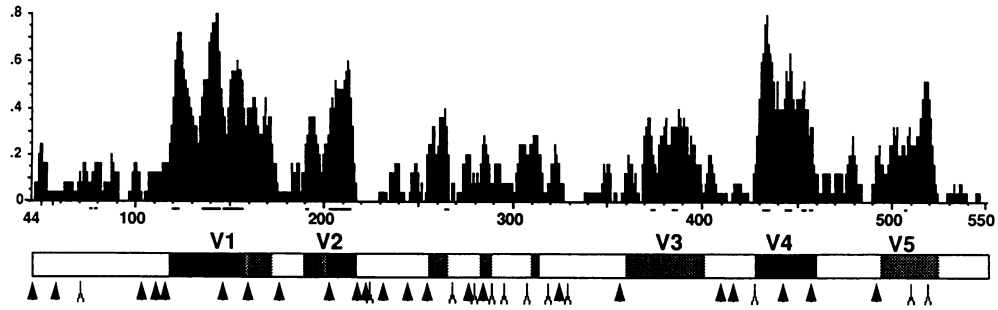

FIG. 5. Localization of the variable domains in the EGP of SIVagm. The deduced amino acid sequence of the SIV EGP from one tantalus monkey (B14) was compared with that of three vervets (5, 20, 34) and one grivet (19). The variability profile is based on the numbers of amino acid substitutions relative to the consensus sequence (12). The profile was smoothed (window of five sites). The numbers on the *x* axis refer to the corresponding amino acid positions in SIVagm EGP. The numbers on the *y* axis represent the percentages of variable amino acids per site. Boxes mark variable regions (V1, V2, V3, V4, and V5). Two types are distinguished: one with 23 to 40% (shaded box) and one with >40% (black box) of nonconserved nucleotides. The lines stand for nucleotide deletions in one or more sequences. Triangles represent conserved cysteine residues, and forks represent conserved potential N-glycosylation sites.

Senegal, a country which suffered greatly from the slave trade, supports this explanation in detecting a significantly lower SIV seroprevalence in young green monkeys and a high rate of variation of SIV distribution from one colony to another (17).

The appearance of species-specific SIVagm groups implies a decreasing frequency of successful cross-species transmission. Indeed, interbreeding these days is limited to some geographic areas, such as Uganda, which is inhabited by both tantalus monkeys and vervets (53). In addition, experimental SIV transmission from one AGM species (tantalus monkey) to another (vervet) seems to be very difficult (33). This is in contrast to the multiple recent and experimentally reproducible cross-species transmissions, such as that of SIVsm in macaques (45) or that of HIV-1 in chimpanzees (22), proposed to explain the origins and relationships of lentiviruses from primates in captivity (29, 50). To study the factors which could have contributed to the decrease of interspecies transmission of SIVagm, more experimental infections of different green monkey species should be done. In addition, more sequence analyses of SIV isolates from different naturally infected AGMs species living in the same geographic area should be performed.

Although probably existing for a very long time in green monkeys, the degree of divergence among SIVagm from a same species is comparable to the intraspecies divergence of other HIVs and SIVs such as HIV-1, HIV-2, and SIVmac, which are pathogenic in humans and macaques (41), respectively, and do not appear to be adapted to their hosts. In other viral systems, nonadapted viruses show a higher level

	consensus	312	CrrPGnKTVLPVTimAGlvFHSQkYNt?LrQAWC 345
tantalus	SIVAGMB05		-----K-------M---------Q---K------
"	SIVAGMB14		----------M---------K------
sabaeus	SIVAGMD29		--I----------A---I--------R-V----
"	SIVAGMD30		----------K------
vervet	SIVAGMTYO		----------MK------
"	SIVAGM155		-K----------R------
"	SIVAGM3		-K----------R---R------
grivet	SIVAGM677		----------R-K----

FIG. 6. Alignments of *env* sequences in SIVagm from four different AGM species and whose location corresponds to that of V3 in HIV-1. Sequences from east African SIVagm were obtained from the Los Alamos sequence data bank (49). The consensus sequence was defined as for Fig. 4. Numbers refer to the amino acid positions in SIVagm(tyo-1) (20).

of genetic variation than adapted viruses (65). In this context, additional studies (4) of genetic variability among sequential virus isolates from naturally infected asymptomatic animals are required. The results presented here provide a more complete description of SIVagm in their respective host species. Further investigations of biological and immunological events that occur during long-term interactions between persistent lentiviruses and their natural hosts should be performed for a better knowledge of AIDS pathogenesis.

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