Simian immunodeficiency virus infection in a patas monkey (*Erythrocebus patas*): evidence for cross-species transmission from African green monkeys (*Cercopithecus aethiops sabaeus*) in the wild

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Socio-ethological studies on troops of African green monkeys (AGMs) (*Cercopithecus aethiops sabaeus*) and patas monkeys (*Erythrocebus patas*) in Senegal have documented physical contacts between these two species. Elevated simian immunodeficiency virus (SIV) seroprevalence rates have been reported for the different AGM subspecies. We report here the extent to which patas monkeys are infected and compare the relatedness of the viruses isolated from these two different species. Among the 85 AGMs and 54 patas monkeys studied, 47% and 7·5%, respectively, had antibodies that cross-reacted with HIV-2 envelope proteins. From two AGMs a virus was isolated. From the patas monkeys, virus isolation was generally not possible, but from one animal that was ill a virus designated pamG31 was amplified by PCR. In addition, for the two SIVagm isolates, an 830 bp region spanning the *env* and *nef* genes was amplified and sequenced. Comparisons of sequences from the *env*/*nef* region revealed 80% identity between pamG31 and SIVagm isolates from AGMs of the *sabaeus* subspecies, and 94% identity between the two SIVagm isolates. Phylogenetic analysis showed that pamG31 belongs to the SIVagm *sabaeus* subgroup. This is the first report of a lentiviral infection in a patas monkey. The close genetic relatedness between pamG31 and SIVagm *sabaeus* viruses is a strong argument in favour of cross-species transmission of SIV between AGMs and patas monkeys in the wild. For these reasons, we propose to refer to this patas virus as SIVagm-pamG31.

Introduction

The origins of human immunodeficiency viruses (HIVs: HIV-1 and HIV-2) remain unclear, but the identification of related lentiviruses in chimpanzees (*Pan troglodytes*) (Peeters *et al.*, 1989, 1992; Huet *et al.*, 1990) and sooty mangabeys (*Cercocebus atys*) (Lowenstine *et al.*, 1986; Hirsch *et al.*, 1989) for HIV-1 and HIV-2, respectively, led to the hypothesis of cross-species transmission from wild-living African non-human primates to humans (Myers *et al.*, 1992; Nathanson *et al.*, 1993; Sharp *et al.*, 1994). This hypothesis is supported by several observations and could explain the pathogenicity of HIVs. With the exception of SIVmac, isolated from macaques in captivity (Daniel *et al.*, 1985; Murphey-Corb *et al.*, 1986; Khan *et al.*, 1991; Novembre *et al.*, 1992), other simian immunodeficiency viruses (SIVs) isolated to date fail to cause disease in their natural host (Fukasawa *et al.*, 1988; Hendry *et al.*, 1986; Ohta *et al.*, 1988; Kraus *et al.*, 1989; Tsujimoto *et al.*, 1989; Hirsch *et al.*, 1993*a*). SIVmac was isolated from different species of captive but not wild-caught macaques and these monkeys, while infected with SIV, develop a fatal immunodeficiency disease, similar to AIDS in humans (Daniel *et al.*, 1985; Khan *et al.*, 1991; King *et al.*, 1990). Because of the close nucleotide similarity between SIVmac and SIVsm, it has been proposed that cross-species transmission occurred between macaques and mangabeys in captivity. The close genetic relationship (80% amino acid identity in Gag proteins) between HIV-2 and SIVsm isolated from

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mangabeys, and the fact that the geographical spread of HIV-2 epidemics corresponds to the natural habitat of sooty mangabeys in West Africa, is an additional argument in favour of cross-species transmission (Marx *et al.*, 1991; Gao *et al.*, 1992).

Based on genomic sequences, five genetically distinct groups have been proposed to classify primate lentiviruses (reviewed in Sharp *et al.*, 1994): (i) HIV-1 and SIVcpz from chimpanzees, (ii) HIV-2 and SIVsm from sooty mangabeys, (iii) SIVmnd from mandrills (*Papio sphinx*), (iv) SIVagm from African green monkeys (*Cercopithecus aethiops*) (AGMs) and (v) SIVsyk from Sykes' monkey (*Cercopithecus mitis*). The SIVagm group can be further subdivided into distinct species-specific virus groups, depending on the geographical origins of the AGM host (Li *et al.*, 1989; Allan *et al.*, 1991; Hirsch *et al.*, 1993*b*; Müller *et al.*, 1993). In fact, the four distinct AGM subspecies, grivets (*C. a. aethiops*), tantalus (*C. a. tantalus*), vervets (*C. a. pygerythrus*) and sabaeus (*C. a. sabaeus*) (Lernould, 1988), harbour equidistantly related viruses. This is a strong argument in favour of long-term infection in these monkeys. Sequence analysis of SIVagm from West African AGMs (Jin *et al.*, 1994*a*) recently revealed a mosaic virus genome structure, consistent with recombination events between ancestors of viruses now found in other AGM subspecies and sooty mangabeys, supporting transmission between different species. Recently, cross-species transmission of SIVagm from AGM to other monkeys living in the same natural habitat has been described: to yellow baboons (*Papio hamadryas cynocephalus*) in Tanzania (Jin *et al.*, 1994*b*) and to African white-crowned mangabeys (*Cercocebus torquatus lunulatus*) in Kenya (Tomonaga *et al.*, 1993).

In Senegal, preliminary results of socio-ethological studies show that AGMs (*sabaeus* subspecies) and patas monkeys (*Erythrocebus patas*) (Galat-Luong *et al.*, 1994*a*, *b*) living in the same ecological habitat have close physical contacts. These include bites and corporeal fluid exchange; such modes of transmission have already been proposed for retroviruses in captive colonies. These observations led us to further examine whether these two different species are infected with an SIV and, if so, to compare SIVs from AGMs and patas monkeys. We report here SIV sequences obtained from a patas monkey and discuss the possible origin of this virus from AGMs.

Methods

Animals. A total of 85 AGMs (*sabaeus* subspecies) and 54 patas monkeys (*E. patas*) were tested for the presence of HIV/SIV antibodies. All of these animals live in the Fathala forest (Saloum Delta National Park, Senegal). They were captured and then anaesthetized for 1 h. Five ml of blood was drawn from each animal. After bleeding, they were released in their natural environment. To the best of our knowledge, all of these monkeys had never been experimentally exposed to SIVs. Among the AGMs, 35 were adults (14 males, 21 females) and 50 were immatures (less than 3 years old) (see Table 1). Fifty-four patas monkeys (21 adults and 33 immatures) were also captured at the same site. With the exception of one animal (see Results), all were in good health and showed no signs of an AIDS-like disease.

Serology. Sera were tested using a commercial HIV-1+2 ELISA (ELAVIA-Mixt; Diagnostics Pasteur, Marnes-la-Coquette, France) and by a line-immunoassay (LIA) on which recombinant proteins and synthetic peptides derived from HIV-1 and HIV-2 were applied (INNOLIA HIV-1+2; Innogenetics NV, Antwerp, Belgium). Positive samples were retested with a commercial HIV-2 Western blot (New Lav Blot II; Diagnostics Pasteur). Positive samples were further confirmed in a radio-immunoprecipitation assay (RIPA) using the SIVagm D30 strain (Müller *et al.*, 1993). Viruses were grown on Molt-4 clone 8 cells (kindly provided by F. Barré-Sinoussi) and metabolically labelled with [^{35}S]methionine overnight at 37 °C (200 µCi/ml at 4×10^6 cells/ml). After collection of the supernatants and cells, the virus was pelleted and then resuspended in lysis buffer (0·02 M-Tris–HCl, pH 7·6, 0·15 M-NaCl, 0·05 M-KCl, 0·001 M-EDTA, 0·0002 M-PMSF, 0·05% aprotinin, 1% β-mercaptoethanol and 2% Triton X-100). The diluted virus (equivalent to 2×10^6 cells or 100 µCi) was then incubated with 10 µl of serum for 1 h at 4 °C in B1 buffer (0·2 M-Tris–HCl, pH 7·6, 0·15 M-NaCl, 0·05 M-KCl, 0·001 M-EDTA, 0·2 mM-PMSF, 0·05% aprotinin, 1% β-mercaptoethanol and 20% glycerol). Immune complexes were adsorbed with Protein A–Sepharose overnight at 4 °C. After washing, immune complexes were eluted in electrophoresis sample buffer containing 1% SDS and β-mercaptoethanol and heated for 3 min at 100 °C. They were then subjected to electrophoresis on a 10% SDS–polyacrylamide gel; [^{35}S]methionine labelled proteins were detected by autoradiography.

Virus isolation. For the two SIVagm strains studied here, viruses were isolated from heparinized whole blood. Plasma was separated by low-speed centrifugation, and the buffy coat, containing peripheral blood mononuclear cells (PBMCs), was recovered. After Ficoll gradient centrifugation, PBMCs were directly co-cultured with Molt-4 clone 8 cells in RPMI containing 10% fetal calf serum, 2 mM-L-glutamine and antibiotics. Co-cultures were supplied with fresh medium every 3–4 days and examined for the appearance of virus-induced cytopathogenicity. Reverse transcriptase (RT) activity in supernatants was used as a marker for virus replication.

PCR. One µg of genomic DNA, prepared independently from spleen and mesenteric lymph nodes for G31 (a patas monkey) and from SIV infected Molt-4 clone 8 cells for P055 and P056 (AGMs), was used as template for PCR amplification. Oligonucleotides (SV6, 5′ GGGAATATCAGCAAGACATTGG 3′; SV4, 5′ CCATCCAGTCCTCCCTTTTCTTT 3′) were designed, from sequences obtained from the D30 sabaeus isolate (Jubier-Maurin *et al.*, 1995), to amplify SIVagm sequences spanning the *env* and *nef* genes (Fig. 1). Another primer pair (SVF, 5′ GCAGTGGACTATGCTGCTTGGAG 3′; 3′LTR, 5′ CAAGTCCCTGTTCGGGCGCC 3′) was used to amplify the long terminal repeat (LTR) core enhancer region (Fig. 1) from pamG31. Reactions were carried out in buffer containing 10 mM-Tris–HCl (pH 9·0), 50 mM-KCl, 1·5 mM-$MgCl_2$, 0·2 mM of each dNTP, 2·5 U of *Taq* DNA polymerase (Promega) and 0·4 µM of each primer. For the *env*/*nef* region, amplification cycles were as follows: an initial denaturing step was carried out at 93 °C for 3 min, followed by 40 additional cycles at 93 °C for 30 s, 54 °C for 30 s, and 72 °C for 90 s (5 min for the last cycle). For LTR amplification, cycling was the same, except for the annealing step performed at 57 °C, and 30 s for the elongation step. PCR products were visualized by ethidium bromide staining after electrophoresis in 1% agarose gels.

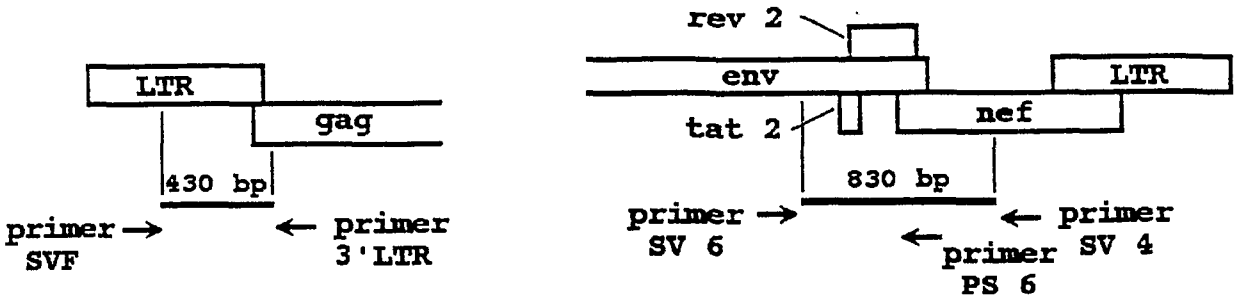

Fig. 1. Locations of the primers used in this study, based on the SIV SAB-1C sequence: SVF, 273–295; 3'LTR, 789–769; SV6, 8423–8445; SV4, 9276–9254; PS6, 8937–8913.

Cloning and sequencing of PCR products. env/nef PCR products (830 bp) for each sample were purified from low-melting-point agarose gels, blunted with DNA polymerase I Klenow fragment and kinased with T4 polynucleotide kinase. They were then ligated into the dephosphorylated *Eco*RV site of M13BM20 (Boehringer Mannheim) and cloned in *Escherichia coli* strain XL1. Positive white clones were expanded and both strands sequenced using the Taq dyedeoxy terminator cycle sequencing kit (Applied Biosystems). Primer PS6 (5' GAGCTCTTGCCACCCATATTCAT 3') was also used to complete the sequence. The LTR PCR product (430 bp) from G31 was purified from a low-melting-point agarose gel and directly sequenced with oligonucleotides SVF and 3'LTR. GenBank accession numbers for the sequences are U26297 to U26300.

Phylogenetic analysis. Nucleotide sequences were aligned, using CLUSTAL (Higgins & Sharp, 1989*a*, *b*), with sequences of previously described SIVagm and other SIV strains. Evolutionary distances were computed using Kimura's empirical two-parameter method. A distance matrix was then used to estimate phylogenetic relationships by the neighbour-joining method (Saitou & Nei, 1987). Reliability of the branching orders was confirmed by the bootstrap approach (Felsenstein, 1985). All analyses were performed using PHYLIP 3.5c (obtained from J. Felsenstein, Dept of Genetics, University of Washington, Seattle, Wash., USA).

Results

Animals and serology

Sera from 85 wild-caught AGMs, belonging to three social groups, were screened for the presence of HIV/SIV cross-reactive antibodies as described in Methods. The frequency of seropositive animals was 47% (40/85) (Table 1). Moreover, age determination showed significant differences between adults and immatures, 83% (29/35) and 22% (11/50) respectively. The sera had antibodies against HIV-2 gp36 and not against HIV-1 proteins, as tested by LIA. Moreover, on commercial Western blots, only antibodies to HIV-2 envelope proteins and not to HIV-1 envelope proteins were observed. The use of RIPA with the SIVagm D30 strain allowed detection of antibodies against the external envelope glycoprotein (gp110) and major viral core protein (p27) (data not shown).

Fifty-four sera from patas monkeys, belonging to four social groups, were also tested by the same methods for the presence of HIV/SIV cross-reactive antibodies. Four animals (7·5%) (Table 1) were found to be seropositive, exhibiting the same reactivity patterns as those observed for AGM sera against HIV-2 envelope proteins by LIA and commercial Western blots, and also p25 by RIPA with antibody raised against SIVagm D30 (data not shown). Among these seropositive monkeys, two (G31 and H5) had lymphadenopathy. G31, a young female, was in very poor physical condition and showed limb paralysis. The animal died a few hours after blood sampling and additional organs were removed for further analysis. H5, a young male, generally seemed to be in good health despite lymphadenopathy. No virus isolation could be performed from PBMC of these patas monkeys because the samples were obtained under field conditions.

Comparison of the sequence from G31 with other SIVs

To further characterize SIVs present in AGMs and patas monkeys, we PCR amplified, cloned and sequenced an 830 bp region spanning the *env* and *nef* genes from samples from one patas monkey and two AGMs, (virus

Table 1. *Incidence of cross-reactive antibodies to HIV-2 in AGMs and patas monkeys*

		Adults			
		Male	Female	Immatures	Total
AGM	No. positive/no. tested	9/14	20/21	11/50	40/85
	Positive (%)	**83**		**22**	**47**
Patas	No. positive/no. tested	1/7	1/14	2/33	4/54
	Positive (%)	**9·5**		**6**	**7·5**

Table 2. *Percentage nucleotide identities in the env/nef region between SIV strains*

	pamG31	SIVagm P055	SIVagm P056	SIVagm SAB-1C	SIVagm 155	SIVagm 677
pamG31	–	80	79	81	64	65
SIVagm P055		–	94	82	65	64
SIVagm P056			–	82	65	64
SIVagm SAB-1C				–	64	65
SIVagm 155					–	63
SIVagm 677						–

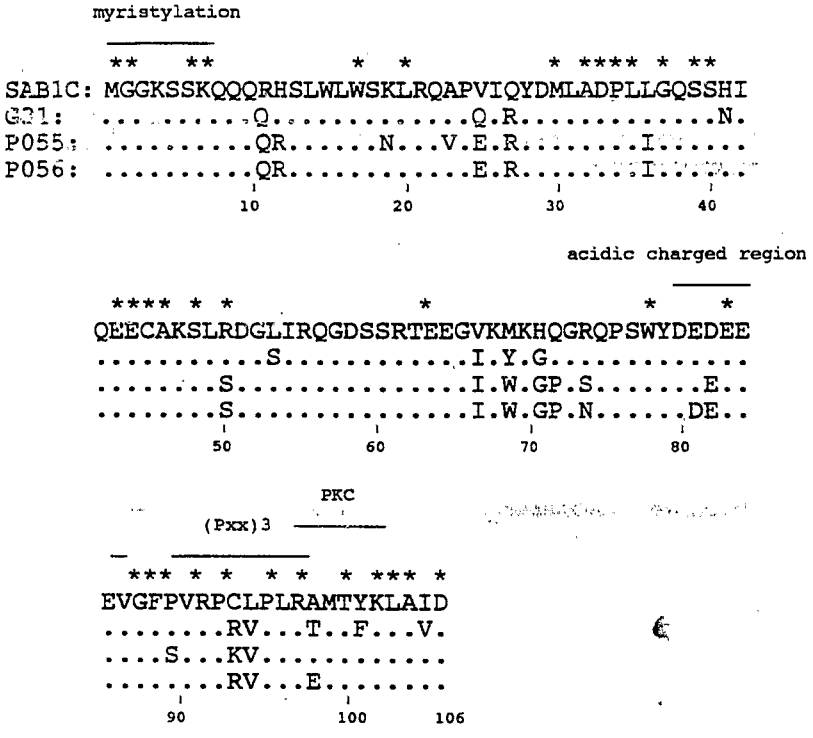

Fig. 2. Alignment of the deduced amino acid sequences of Nef proteins with SIVagm SAB-1C Nef. An asterisk (*) indicates amino acids conserved among all SIVagm. The myristylation signal, acidic charged region, (Pxx)3 repeat sequence and putative PKC phosphorylation site are indicated.

strains designated pamG31, and P055 and P056, respectively, in the figures and tables) (Fig. 1). No amplification was detected when using PBMC DNA from seronegative animals of the two monkey species. Sequences were then aligned and compared to previously described SIVs (Myers *et al.*, 1994). Sequence identity scores are shown in Table 2. No significant differences were observed between spleen and lymph node PCR product sequences (98% identity, data not shown) for G31, and they were closely related to sequences obtained for P055 and P056 (approximately 79% identity). These latter sequences were quite similar (94% identity), which is not surprising as they were amplified from animals belonging to the same troop. Moreover, the presence of a *nef* ORF in all sequences confirmed their retroviral origin.

To gain further insight into the relationships between these different viruses, deduced amino acid sequences for Nef were compared with the recently described SIVagm SAB-1C sequence (Jin *et al.*, 1994*a*). As shown in Fig. 2, conserved amino acids of AGM Nef proteins were also observed in the region studied, with the exception of the proline residue at position 89 replaced by serine in P055, and the tyrosine residue at position 101 replaced by a phenylalanine in pamG31. Compared to HIV-1 (Shugars *et al.*, 1993), with the exceptions listed above, the

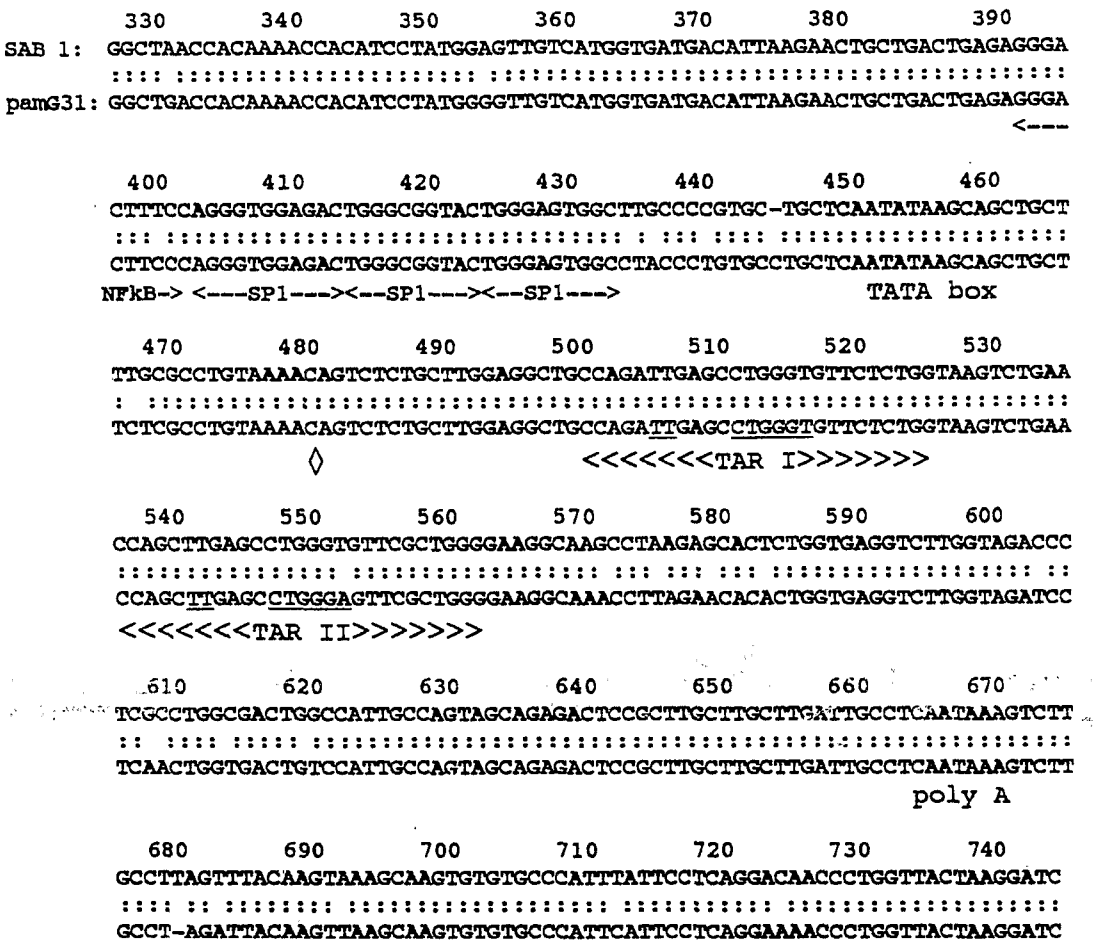

Fig. 3. Alignment of LTR nucleotide sequences from G31 and SIV SAB-1C. Numbers above the sequences refer to nucleotide location in the SIV SAB-1C complete sequence referenced in the database. Highly conserved regulatory elements are indicated: NFκB and SP1 sites, TATA box, transactivation response element (TAR I and II) and polyadenylation signal. The symbol (◇) indicates the putative mRNA start. Bulge and loop sequences in the putative TAR are underlined. The symbol (:) indicates identical nucleotides in the two sequences.

myristylation signal, acidic charged region, (Pxx)3 repeat sequence (only three repetitions in SIVagm; Myers *et al.*, 1994) and putative PKC phosphorylation site were also well conserved. In contrast with HIV-1, amino acids following the myristylation signal seemed much less variable.

The LTR sequence obtained from pamG31 was aligned to SIVagm SAB-1 (Fig. 3*a*) and compared with four SIVagm sequences from the *sabaeus* subspecies. These sequences were very similar (approximately 94% identity; data not shown), with regulatory elements, including the putative NFκB and SP1 sites, TATA region, transactivation response element (TAR) and polyadenylation sites all highly conserved. As recently reported for SIVagm from the *sabaeus* subspecies (Jin *et al.*, 1994*a*), duplication of the TAR element was also found for this patas virus. When compared with each other, sequences of SIVagm from the *sabaeus* subspecies were also highly similar (approximately 96% identity), indicating low variability for this part of the LTR. Taken together, these results suggest that the virus present in G31 is related to viruses described in AGMs of the *sabaeus* subspecies.

Phylogenetic relationships between pamG31 and other SIVs

To determine the evolutionary relationships among these new viruses and other lentiviruses, a phylogenetic tree was constructed from the *env/nef* nucleotide sequences. These sequences were aligned with sequences from four of the five groups used for lentiviral classification (HIV-1 group not included). The branching order (Fig. 4) clearly showed that the SIV present in G31 belonged to the SIVagm *sabaeus* group, along with P055, P056 and SAB-1. These results also confirmed that SIVagm from

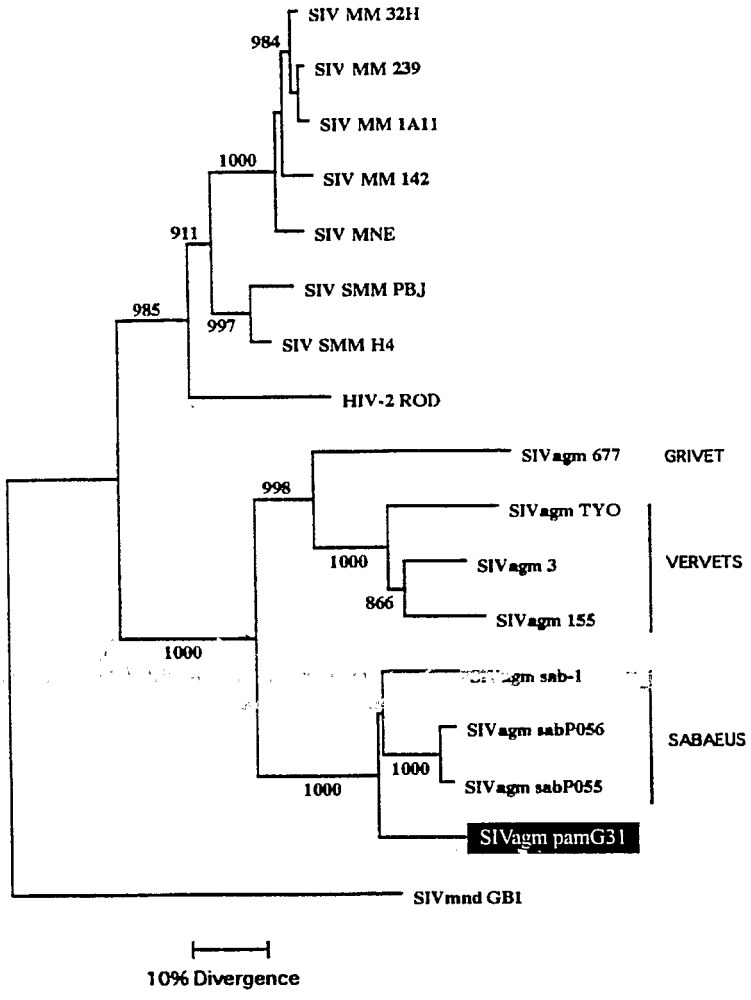

Fig. 4. Rooted neighbour-joining tree for the *env/nef* region nucleotide sequences (802 sites). SIVmnd GB1 serves as an outgroup. The numbers alongside the branch points indicate the number of times the group consisting of the species which are to the right of that point occurred in more than 80% of the trees.

the *sabaeus* subspecies form a separate subgroup among primate lentiviruses, distinct from the other SIVagm isolates. Identical branching order and high bootstrap values were also obtained when a phylogenetic analysis was performed using deduced amino acid sequences for the *env* gene (data not shown).

Discussion

We report data concerning wild-living troops of AGMs and patas monkeys, from Senegal, for which SIV seroprevalence was determined and a partial virus characterization was performed. The serological results showed significant differences in SIV seroprevalence between AGMs and patas monkeys: 47% for AGMs, which is consistent with already published data for other AGM subspecies (Hendry *et al.*, 1986; Lowenstine *et al.*, 1986; Ohta *et al.*, 1988; Allan *et al.*, 1991; Hirsch *et al.*, 1993*b*; Müller *et al.*, 1993) and 7·5% for patas monkeys. For AGMs, significant differences in seroprevalence between adults and immatures were also observed, 83% and 22% respectively, indicating that sexual activity is the major route of transmission of SIVagm in the wild. Similar conclusions have been published for wild-living AGM populations belonging to the grivet subspecies (Phillips-Conroy *et al.*, 1994). For patas monkeys, the number of positive animals was too low to draw any conclusions on the mode of transmission since two of the four seropositive animals were adults and two were immatures.

We present here, for the first time, evidence of SIV infection in patas monkeys, and a genetic characterization of this new virus. Sequence data for a region spanning the *env* and *nef* genes obtained from one seropositive female, (G31) were compared to sequences obtained from two seropositive AGMs living close to this patas monkey. Close physical contact, such as bites and grooming (A. Galat-Luong, personal observation), were noted between AGMs and patas monkeys. Sequence comparisons showed that the virus infecting G31 (pamG31) was related (approximately 80% identity) to SIVagm isolates from AGMs living in close proximity. The same similarity was found for the viruses we describe here (from AGMs and patas monkey) when compared to the

SIVagm SAB-1C isolated from a Senegalese AGM whose exact geographical origin is unknown. In comparison, the two SIVagm isolates described here were closely related (94% identity). Taken together, these results and others (F. Bibollet-Ruche, unpublished results) suggest that, in AGMs, a major SIVagm variant might be present in troops living in close contact.

A highly conserved LTR sequence was found in pamG31 when compared to SIVagm isolates from the *sabaeus* subspecies (94% identity), including TAR duplication which was found only in this subtype. One would expect, in the case of cross-species transmission, that there might be selection pressures on structural and regulatory genes rather than on regulatory elements. However, the rate of evolution of SIVagm has not been quantified. A study by Michael *et al.* (1994), concerning naturally occurring HIV-1 LTR genotypes, demonstrated limited variability during the course of infection. In the case of the acutely lethal SIVsmmPBj strain, a limited role of LTR sequences as disease determinants has been reported (Novembre *et al.*, 1993). The high similarity of the LTR sequence from pamG31 with SIVagm *sabaeus* isolates is a strong argument in favour of cross-species transmission from AGM to patas monkeys, but cannot be used to evaluate the divergence time between these viruses. As expected from the sequence comparisons, phylogenetic analysis with *env*/*nef* sequences confirmed that these viruses, including that from the patas monkey, belong to the SIVagm *sabaeus* group, and argue in favour of cross-species transmission from AGM to patas monkeys. Here again, the relatively short distances between the viruses in this group did not allow evaluation of the transmission time. To further characterize SIV infection in this species, we propose to refer to this patas monkey virus as SIVagm-pamG31. Similar results were recently described for infections of yellow baboon (Jin *et al.*, 1994*b*; Kodama *et al.*, 1989) and African white-crowned mangabeys (Tomonaga *et al.*, 1993) by SIVagm-related viruses. However, despite the apparent active replication of these viruses *in vivo*, as suggested by high-titres of SIVagm-specific antibodies, no evidence of illness was observed in these primates.

For the last decade, SIVs isolated from naturally infected monkeys were thought to be nonpathogenic for their natural hosts, as a result of co-evolution. A study by Norley *et al.* (1990) showed the roles of both host and viral determinants for apathogenicity of SIVagm in AGMs. Only a limited number of cases of lethality were reported for infection by SIVagm in other experimentally infected monkey species (Gravell *et al.*, 1989; Johnson *et al.*, 1990). Recently, rapid induction of AIDS by a molecular SIVagm clone was observed in pig-tailed macaques but not in rhesus macaques (Hirsch *et al.*, 1995). Early clinical signs of the disease include weight loss, failure to thrive, chronic diarrhoea and lymphadenopathy.

In the case of G31, a female patas monkey, clinical signs noted before death (lymphadenopathy and leanness) favoured the hypothesis that SIVagm is pathogenic in patas monkeys, but we could not determine whether these clinical signs were due to SIV infection. In fact, we frequently noticed lymphadenopathy in healthy wild-living monkeys (AGMs and patas monkeys). We were not able to link these clinical observations to haematologic parameters (such as leukocyte count or CD4/CD8 ratio), since the blood samples were obtained under field conditions. The lower SIV prevalence in patas monkeys was also an argument in favour of pathogenicity. If SIVagm in this unusual host is pathogenic, only recently infected monkeys or survivors infected by a non-pathogenic strain would be detected. Another explanation, which is not exclusive, is the different social organization of AGMs and patas monkeys. In the latter, troops are characterized by a single reproductive dominant male for several females. In contrast, in AGM troops, several males can have sexual contacts with several females, which is more favourable for the spread of SIV. However, we cannot exclude the possibility of a species-specific SIV in patas monkeys, as *Erythrocebus* (which comprises only the patas monkey) and *Cercopithecus* are genetically closely related (Dutrillaux, 1988). Further field observations are necessary to confirm this hypothesis.

Among naturally infected monkeys, AGMs are thought to have been infected for a long time because of the high seroprevalence rates and genetic diversity in the SIVagm group. Moreover, the mosaic genome structure of SIV present in West African AGMs suggests ancient cross-species transmission between sooty mangabeys and AGMs. Following the description of infection of yellow baboons (Jin *et al.*, 1994*b*) and African white-crowned mangabeys (Tomonaga *et al.*, 1993), this third SIVagm-related virus, described here for a patas monkey, confirms the hypothesis that *Cercopithecus* is a major source of SIV infection for other non-human primates. Low seroprevalence rates were noted in both cases, indicating that horizontal transmission is weak between animals belonging to the recipient species. However, these cross-species transmission events, rare or poorly detected in the wild, seem to be quite recent as compared to AGM infection for which speciation has given rise to four distinct subspecies-specific viruses.

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