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Simian immunodeficiency viruses (SIVs) from eastern and southern Africa: detection of a SIVagm variant from a chacma baboon

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Simian immunodeficiency viruses (SIVs) have been shown to infect many Old World African primate species. Thus far, no work has been published on southern African primates. In this study we investigated the genetic diversity between SIV strains from Kenyan and South African vervets (*Cercopithecus aethiops pygerythrus*). We amplified and sequenced a 1113 bp region of the *env* gene. Phylogenetic analysis of these sequences showed that all strains clustered with members of the vervet subgroup of SIVagm. The SIVs from South African vervets differed by 7% from each other and by 8–14% from the Kenyan SIV strains, while the Kenyan SIV strains differed by 10–21% from SIVagm of other east African vervets. We also isolated and sequenced, for the first time, a SIV strain from a healthy chacma baboon (*Papio ursinus*), caught in South Africa. Phylogenetic analysis of the *env* region showed the virus to be closely related to the South African vervet SIV strains, while analysis of its *pol* region confirmed the virus to be a SIVagm variant.

The study of genetic diversity in African lentiviruses is an ongoing process which started more than a decade ago. Interest was sparked by the discovery of human immunodeficiency virus type 1 (HIV-1) and later HIV-2. Simian immunodeficiency viruses (SIVs) have been shown to infect (without inducing disease) two of the three Old World African primate families: the Cercopithecidae and Pongidae. Viruses have been isolated and/or sequenced from the following

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Cercopithecidae monkeys: the four species of *Cercopithecus aethiops* [vervets (Fukasawa *et al*., 1988; Allan *et al*., 1990), grivets (Allan *et al*., 1990; Fomsgaard *et al*., 1991), sabaeus (Allan *et al*., 1991; Bibollet-Ruche *et al*., 1997) and tantalus (Müller *et al*., 1993)]; Sykes' monkeys (Hirsch *et al*., 1993); two types of mangabey monkeys – sooty mangabeys (*Cercocebus atys*) (Fultz *et al*., 1986; Hirsch *et al*., 1989; Lowenstine *et al*., 1986) and white-crowned mangabeys (*Cercocebus torquatus lunulatus*) (Tomonaga *et al*., 1993); as well as from two baboon species – mandrills (*Papio sphinx*) (Tsujimoto *et al*., 1988, 1989) and one yellow baboon (*Papio cynocephalus*) which became infected via cross-species transmission (Jin *et al*., 1994*a*).

In the family Pongidae, virus has so far only been isolated from a chimpanzee (Peeters *et al*., 1989, 1992). In the third family, Hominidae, to which *Homo sapiens* belongs, infection is almost invariably followed by acquired immunodeficiency syndrome (AIDS) and death. This is also true for Old World monkeys such as macaques, naturally occurring mostly outside Africa. Virus transfer from African monkeys to Asian macaque species resulted in an AIDS-like illness in these simians (Hirsch *et al*., 1989; Murphey-Corb *et al*., 1986; Novembre *et al*., 1992).

Most of the studies on SIVs concentrated on animals in various regions of tropical Africa. The picture is not complete without also investigating SIVs from southern African primates. In this study we investigated six animals with HIV/SIV-cross-reactive antibodies: three vervets (*Cercopithecus aethiops pygerythrus*) from Kenya (IPR806, IPR859, IPR1185), and two vervets (ZA358, ZA40) and a chacma baboon (*Papio ursinus*) (Bab590) from South Africa. All animals were in good health and showed no clinical signs of an AIDS-like related disease at the time of trapping and sampling. The Kenyan vervets were kept in separate cages in breeding colonies at the Institute of Primate Research in Nairobi. Two of the animals (IPR806 and IPR859) were born in captivity from the same parents. The South African animals were wild-caught primates (ZA358 from the Free State Province and ZA40 from the Eastern Cape Province) while Bab590 was captured in the Western Cape Province. The baboon was kept in an animal facility at Cape Town for 4 years before it was sacrificed.

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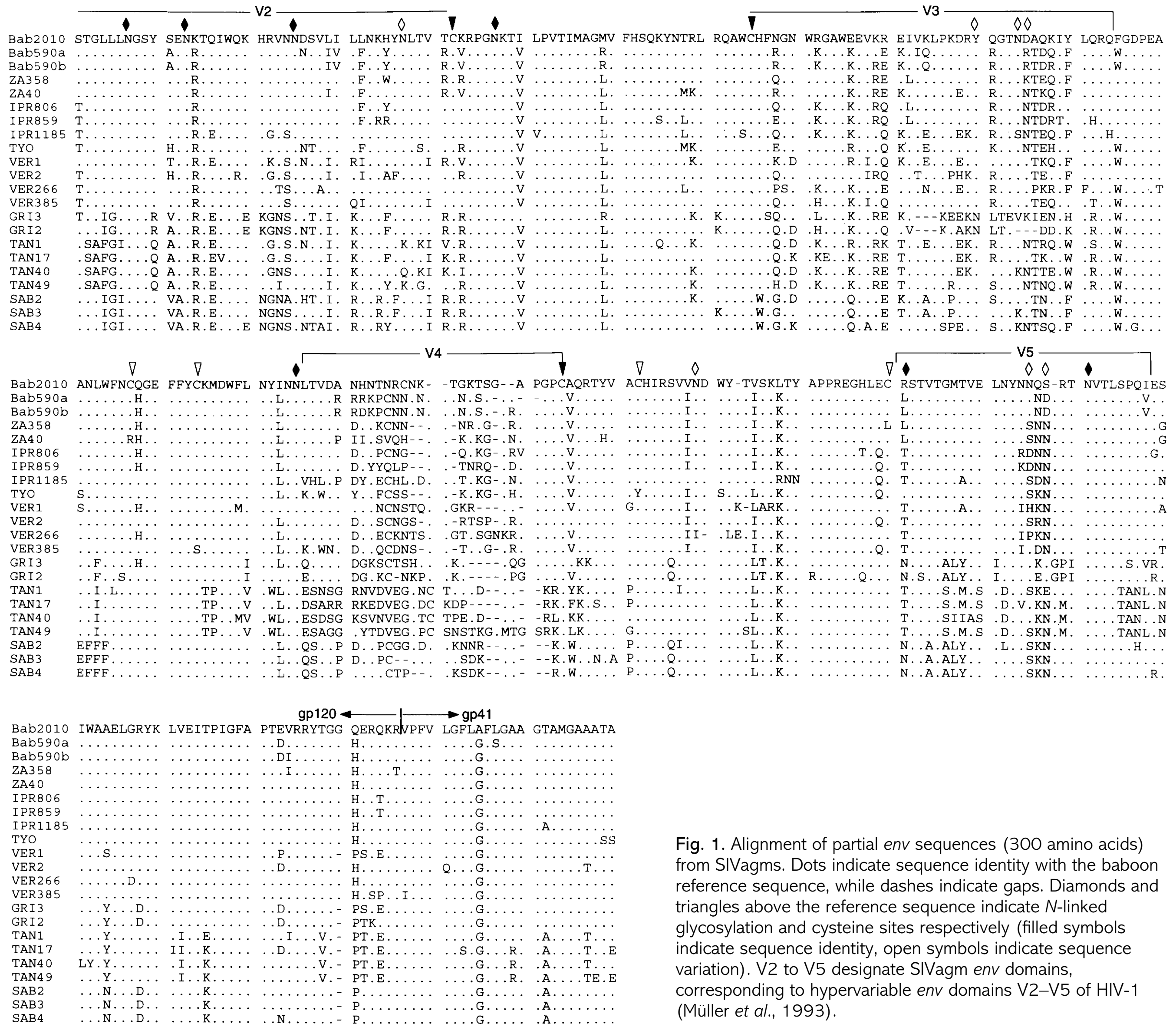

Fig. 1. Alignment of partial *env* sequences (300 amino acids) from SIVagms. Dots indicate sequence identity with the baboon reference sequence, while dashes indicate gaps. Diamonds and triangles above the reference sequence indicate *N*-linked glycosylation and cysteine sites respectively (filled symbols indicate sequence identity, open symbols indicate sequence variation). V2 to V5 designate SIVagm *env* domains, corresponding to hypervariable *env* domains V2–V5 of HIV-1 (Müller *et al.*, 1993).

Blood from the vervets was drawn into EDTA tubes and cell lysates were prepared for PCR from peripheral blood mononuclear cells (PBMCs) as previously described (Albert & Fenyö, 1990). Virus culture from the PBMCs of Bab590 was attempted by growing the cells in RPMI 1640 (Sigma) supplemented with 20% heat-inactivated foetal calf serum, antibiotics, L-glutamine and 1% phytohaemagglutinin (Wellcome Diagnostics). After 2 to 3 days, 2% Lymphocult T-HP (Biotest) was added and the PBMCs were then cocultivated (1:1) with CEM-SS cells (Nara *et al.*, 1987; Nara & Fischinger, 1988). Cultures were monitored for cytopathic effect and a lentivirus infection was confirmed by monitoring the culture supernatants for reverse transcriptase (RT) activity as previously described (Rubsamen-Waigman *et al.*, 1986). Total cellular DNA was extracted for PCR from 10^8 RT-positive cultured lymphocytes from two specimens (Bab590a and Bab590b) by the standard phenol–chloroform method (Sambrook *et al.*, 1989).

A nested *env* PCR (outer primer set: *env* A 5′ GAAGCTTGTGATAAAACATATTGGAT; *env* B 5′ AGAGCTGTGACGCGGGCATTGAGG; inner primer set: *env* C 5′ GTGCATTGTACAGGGTTAATGAATACAACAG; *env* D 5′ TTCTTCTGCTGCAGTATCCCAGCAAG) was used to amplify sequences from the primary uncultured PBMCs of the five vervets and cultured PBMCs from two Bab590 specimens (Jin *et al.*, 1994*a*). A short region of the *pol* gene was also amplified from Bab590 using the universal primers UNIPOL1 (5′ AGTGGATTCATAGAAGCAGAAGT) and UNIPOL2 (5′ AAAATTTTCTTCCCCTCCTTATCCCC) as described (Miura *et al.*, 1990).

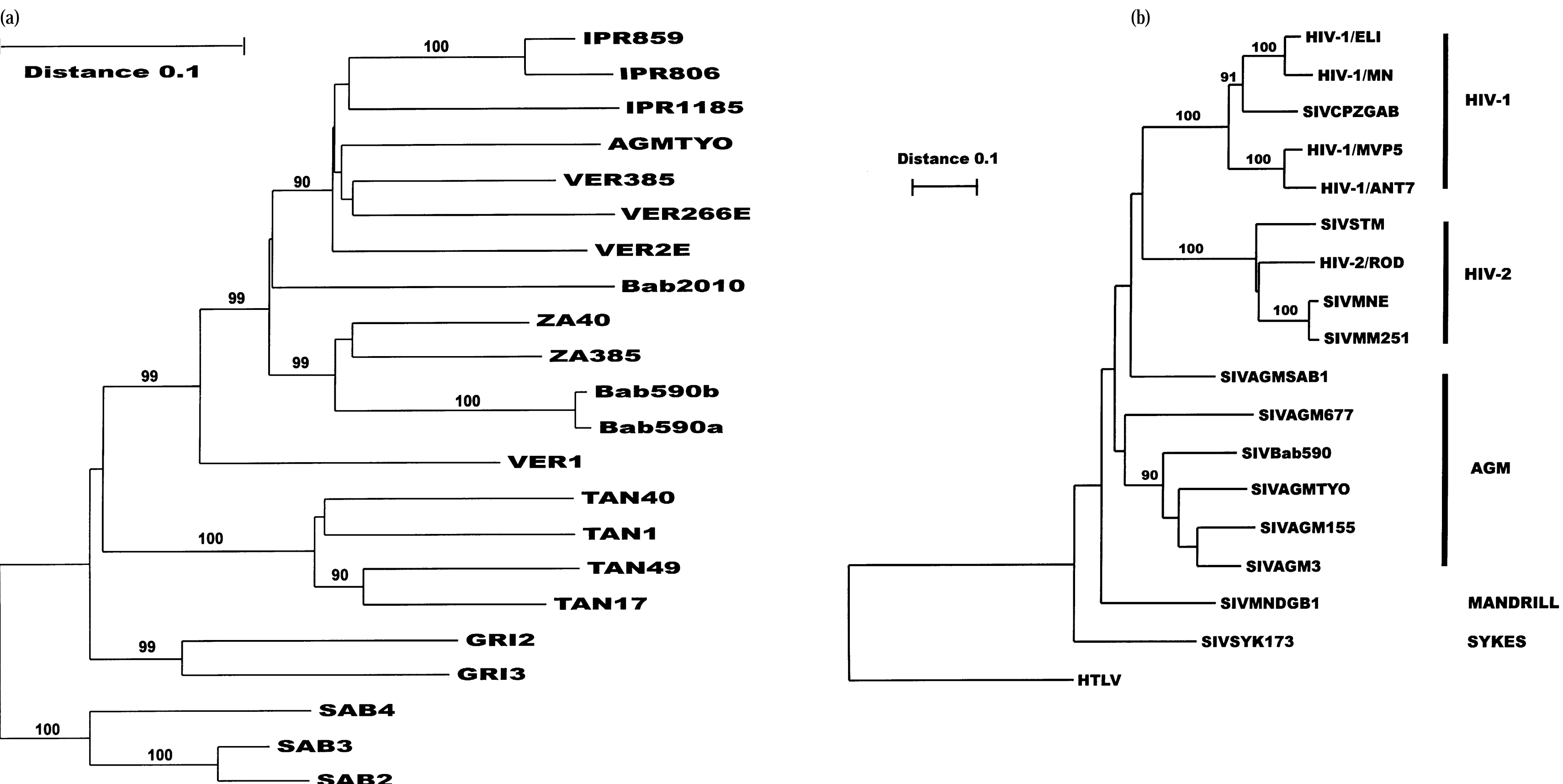

Fig. 2. (*a*) Unrooted phylogenetic tree analysis of different South African and Kenyan SIVagm isolates. The neighbour-joining method for tree construction is based on 1113 bp of the *env* gene. An indication of the degree of sequence dissimilarity is shown on the horizontal axis. The virus strains are indicated on the vertical axis. Sequences of the reference strains were obtained from GenBank: Bab2010 (U10897) (Jin *et al.*, 1994*a*); TYO-1 (X07805) (Fukasawa *et al.*, 1988); Ver1 (U04003) (Johnson & Hirsch, 1991); Ver2 (U04004) (Allan *et al.*, 1990); Ver3 (M30931) (Baier *et al.*, 1989); Ver266 (U10896), Ver385 (U10898), Tan1 (U03999), Tan17 (U034000), Tan40 (U034001), Tan49 (U034002) (Jin *et al.*, 1994*b*); Gri1 (Ver677) (M66437) (Fomsgaard *et al.*, 1991); Gri2 (U-3995), Sab2 (U03996), Sab3 (U03997), Sab4 (U03998) (Allan *et al.*, 1991). (*b*) Neighbour-joining phylogenetic tree analysis of Bab590 compared with published HIV-1, HIV-2, SIVagm, SIVmnd and SIVsyk *pol* sequences. The tree was rooted with human T-lymphotropic virus (HTLV) and based on a 227 bp region of the *pol* gene. In both (*a*) and (*b*) the number of bootstrap trees out of 100 replications supporting a particular phylogenetic group by more than 90% is placed alongside the node considered.

Table 1. Amino acid sequence identity among Env proteins of SIVagm lentiviruses

	TYO	VER-1	VER-2	VER266	VER385	GRI-1	GRI-2	TAN1	TAN17	TAN40	TAN49	SAB-2	SAB-3	SAB-4	IRP806	IRP1185	IRP859	ZA358	ZA40	BAB2010	BAB590A	BAB590B
TYO		17%	15%	15%	13%	28%	28%	29%	31%	31%	28%	24%	24%	26%	13%	16%	14%	13%	13%	15%	15%	15%
VER-1			18%	18%	16%	27%	26%	26%	27%	26%	27%	23%	23%	23%	18%	19%	21%	17%	15%	19%	16%	17%
VER-2				15%	13%	27%	26%	28%	27%	28%	28%	24%	22%	25%	12%	14%	14%	12%	13%	14%	15%	14%
VER266					12%	28%	28%	30%	29%	29%	29%	27%	25%	26%	11%	13%	12%	12%	12%	15%	13%	13%
VER385						29%	27%	29%	28%	28%	28%	27%	25%	27%	10%	13%	12%	12%	14%	14%	15%	15%
GRI-1							13%	30%	28%	28%	29%	25%	24%	24%	28%	28%	30%	27%	27%	28%	25%	25%
GRI-2								29%	28%	28%	30%	24%	24%	22%	26%	27%	28%	26%	28%	26%	24%	25%
TAN1									13%	12%	12%	26%	27%	28%	30%	27%	31%	28%	28%	30%	28%	28%
TAN17										12%	12%	28%	27%	29%	28%	27%	30%	28%	28%	30%	27%	27%
TAN40											11%	27%	27%	30%	29%	27%	31%	29%	28%	29%	29%	28%
TAN49												26%	27%	29%	27%	28%	28%	26%	26%	28%	26%	26%
SAB-2													5%	7%	27%	25%	28%	24%	24%	25%	24%	25%
SAB-3														7%	26%	24%	27%	23%	23%	23%	24%	24%
SAB-4															27%	26%	29%	25%	25%	26%	24%	25%
IRP806																13%	5%	8%	11%	12%	10%	9%
IRP1185																	15%	14%	14%	15%	17%	17%
IRP859																		10%	12%	15%	13%	12%
ZA358																			7%	12%	9%	8%
ZA40																				14%	9%	9%
BAB2010																					14%	14%
BAB590A																						2%
BAB590B																						

PCR products were cloned by using the PCR-Script SK(+) cloning kit (Stratagene) according to the manufacturer's protocol. After transformation and identification of positive recombinant clones, plasmids were purified on anion-exchange columns (Qiagen) and plasmid DNA was sequenced using the Sequenase version 2.0 sequencing kit (USB). Nucleotide sequences were aligned by the Clustal V software program (Higgins & Sharp, 1989) and compared with previously published SIV sequences from different geographical regions. Distance calculation, tree construction methods and bootstrap analysis were done with the MEGA (Kumar *et al.*, 1993) and TREECON (van de Peer & Wachter, 1993) software packages. The sequences have been deposited in the GenBank database under accession numbers AF015903–AF015909.

The amino acid sequences corresponding to 1113 bp of the studied vervet and baboon SIV strains spanning the V2 to V5 domains of gp120 and the first 24 amino acids of the transmembrane protein were compared with previously described *env* sequences of SIVs isolated from other vervets and baboons (Allan *et al.*, 1990, 1991; Baier *et al.*, 1989; Fukasawa *et al.*, 1988; Hirsch *et al.*, 1993; Jin *et al.*, 1994*a*, *b*; Johnson & Hirsch, 1991) (Fig. 1). Sequences from Bab590 showed conserved and variable regions similar to those reported for vervet, grivet, tantalus and sabaeus viruses. In contrast to the hypervariable V3 loop of HIV-1, the corresponding region of our SIVagms showed very little sequence variability. The results were similar to those of other investigators (Burns & Desrosiers, 1991). The cysteine residues tended to be conserved, but potential *N*-glycosylation sites were only conserved in the second and fifth variable (V2 and V5) regions. The functional CD4 binding domain and the envelope glycoprotein precursor cleavage sites were also conserved. Vervet ZA40 showed a cysteine residue loss between the V3 and V4 regions, while in vervet ZA358 the loss was immediately before the V5 region. The loss of these cysteine residues might affect the disulfide bonding of the membrane proteins of these strains, which in turn could influence antigenicity (Gregory *et al.*, 1991).

Phylogenetic analysis of the *env* sequences revealed four major branches containing sequences representative of the four different subtypes of AGMs: vervets, grivets, sabaeus and tantalus monkeys (Fig. 2*a*). All of the South African SIV sequences, including the two clones from Bab590, clustered with members of the vervet subgroup of SIVagm. The South African types formed a statistically significant cluster within the vervet subtype, supported by 100% of the bootstrap trees. Five major branches were observed after phylogenetic analysis of the *pol* region, indicating five distinct lineages of primate lentiviruses: HIV-1/SIVcpz, HIV-2/SIVsm/SIVmac, SIVmnd (mandrill), SIVsyk (Sykes' monkeys) and SIVagm (African green monkeys) (Sharp *et al.*, 1994). Bab590 clustered with the other AGMs as expected (Fig. 2*b*).

Intrasubtype *env* sequence was calculated from a Kimura distance matrix, and distances were at a maximum of 31%. The South African vervet strains differed by 7% from each other, 8–14% from the Kenyan vervets, 12–17% from other east African vervets and 8–9% from the two Bab590 strains (Table 1). The two clones from Bab590 differed by 2% from each

other and by 14% from the yellow baboon previously described. The Kenyan vervet strains differed by 5–15% from one another, 9–17% from the chacma baboon and 10–21% from other east African vervets. Of significance is that the South African and Kenyan vervet strains differed less than the Kenyan SIV strains did compared with other east African vervet strains (Table 1).

The baboon appeared healthy during its years in captivity. This might have been due to the presence of the virus in this species for a long time-period, similar to that of AGMs. Another possibility is that the baboon could have been infected by cross-species transmission from vervets before it was captured. Monkeys and baboons share the same geographical distribution in most parts of southern Africa, except in a large part of the Western Cape Province where only baboons occur (Smithers, 1983). It could also have been a cross-species transmission from a vervet to a chacma baboon in a region outside the Western Cape, with subsequent intra-species baboon-to-baboon transmission. The origin of the baboon's infection remains speculative and the possible transmission of SIVs between monkeys and baboons in the Southern Africa region is still to be determined. In order to compile a more complete picture, more specimens from different geographical areas should be collected to shed light on the question.

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