A novel simian immunodeficiency virus isolated from a Schmidt’s guenon (*Cercopithecus ascanius schmidti*)

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A novel simian immunodeficiency virus (SIV) was characterized from a Schmidt's guenon (*Cercopithecus ascanius schmidti*), which was housed in a local zoo. The virus infection was detected during a routine serological screening for antibodies that were cross-reactive with SIVmac antigens. Infection with an immunodeficiency virus was confirmed using an INNO-LIA HIV Confirmation assay. Using DNA isolated from a blot clot, a 1895 nt partial *pol* sequence was amplified and sequenced. Phylogenetic analysis showed that this virus, designated SIVschm, shares a distant relationship with SIVgsn, isolated from greater spot-nosed monkeys, and is one of the most divergent SIVs identified to date.

Since the discovery of human immunodeficiency virus type 1 (HIV-1) as the causative agent of AIDS, much effort has been invested to characterize the zoonotic reservoir of this new human pathogen (Hahn et al., 2000). Closely related immunodeficiency viruses have been described in many species of African nonhuman primates (simian immunodeficiency virus, SIV). Amongst these SIVs, the one that is most closely related to HIV-1 is SIVcpz, a virus isolated from chimpanzees (Gao et al., 1999; Peeters et al., 1989). The origin of the AIDS epidemic in man has been estimated to have been initiated in the first half of the 20th century (Korber et al., 2000) and is likely to have been started by multiple zoonotic transmissions of SIVcpz to humans (Gao et al., 1999). However, because of the low prevalence of SIVcpz in wild chimpanzees (Santiago et al., 2003), it remains possible that other, as yet unknown, viruses may be the ultimate reservoirs of the ancestors of HIV-1. In contrast to HIV-1, it has been suggested for HIV-2 that this human virus is the result of zoonotic transmissions of SIV from sooty mangabeys (Gao et al., 1992). Clearly, SIVs that occur naturally in African monkeys and great apes are a real risk for new zoonotic transmissions of immunodeficiency viruses into the human population. Recently, Peeters et al. (2002) reported on a comprehensive study of SIV infections in monkeys from Cameroon that were hunted for bushmeat or were kept as pets. The high incidence of SIV infection in nonhuman primates (16-6%) suggests that, in Africa, exposure of humans to a variety of SIVs must be a relatively common event. Here we report on the discovery of a new SIV variant from a Schmidt’s guenon (*Cercopithecus ascanius schmidti*, SIVschm), a subspecies of the red-tailed guenon (*C. ascanius*).

During a screening of serum samples from various nonhuman primates for antibodies against SIV, we detected a Schmidt’s guenon that had serum antibodies that reacted strongly with SIVmac antigens used in a SIV antibody ELISA (Warren et al., 1998). The serum sample of this animal, Qu, was analysed further using the INNO-LIA HIV Confirmation assay (Innogenetics) and the diagnosis of infection with an immunodeficiency virus was confirmed (Fig. 1, lane 1). In this assay, the serum sample cross-reacted strongly with the HIV-2 gp36 and HIV-1 p24 proteins and interacted weakly with HIV-1 gp41. This pattern of reactivity is comparable to that observed with sera of other African monkeys (Courgnaud et al., 2001; Peeters et al., 2002).

To molecularly characterize this virus, we extracted DNA from the only available blot clot of this animal. Using the Puregene DNA Isolation kit (Gentra Systems), sufficient DNA was isolated to be used as a template for PCR. Despite the poor quality of the DNA, we were able to amplify a 163 nt fragment located in the *pol* gene using the published primer set DR4/DR5 (Clewley et al., 1998). This fragment was then used to design two SIVschm-specific primers (SIVschm-1, 5'-CATAGTGGACATGAAGGATGCCTTTTACAGCGTC-3', and SIVschm-2, 5'-GGAGAGCGCTATCAGTTCAGTCTTG-3'), which were combined with the primer Unipol2 (Miura et al., 1990) in a semi-nested amplification reaction.

Both amplification reactions were performed in a 50 μl volume using 10 μl DNA, 10 mM Tris/HCl (pH 8.3),...
50 mM KCl, 0.01% BSA, 50 pmol each primer, 0.2 mM each dNTP, 2 mM MgCl$_2$ and 2.5 units AmpliTaq Gold (PE Applied Biosystems). Samples were preheated for 15 min at 94°C to activate the enzyme and then cycled for 20 s at 94°C, 20 s at 55°C and 2 min at 72°C for 35 rounds of amplification. PCR products were isolated from agarose gel using the QIAquick Gel Extraction kit (Qiagen) and sequenced directly using the ABI PRISM BigDye Terminators V3.0 Cycle Sequencing kit on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems). Sequence analysis was performed using the Seqman II software package (DNASTAR).

In total, a 1895 nt partial pol sequence was amplified and sequenced. The fragment (EMBL accession no. AJ551401) was aligned to published SIV pol sequences using Se-Al Sequence Alignment Editor, version 2.0a11 (Rambaut, 2002) and all sites containing a gap were excluded from analysis. Genetic distances between the newly derived SIV and SIVs representing known lineages were calculated using Kimura’s two-parameter method and a phylogenetic tree was constructed using the neighbour-joining method, as implemented in the PAUP, version 4.0b10, software package (Swofford, 2002).

Genetically, the SIVschm pol sequence was highly divergent from other SIVs identified to date (Table 1). Overall genetic distances from other SIVs were comparable to those of SIVcol, from guereza colobus monkeys, and SIVolc, from olive colobus monkeys, which are among the most divergent SIVs described to date (Courgnaud et al., 2001, 2003). The most closely related to SIVschm was SIVgsn, from greater spot-nosed monkeys (Courgnaud et al., 2002), which had

![Fig. 1. INNO-LIA HIV Confirmation assay on a serum sample from a Schmidt's guenon. The assay was performed according the manufacturer's instructions. Samples were considered positive when they gave a positive result with at least two HIV antigens. Lanes: 1, C. ascanius schmidti Qu; 2, SIVmac-infected macaque; 3, SIVcpz-infected chimpanzee; 4, negative control; 5, positive control.](image-url)
47.4% genetic distance from the new virus. Direct comparison of the deduced amino acid sequences of the pol gene sequences confirmed its relatedness with SIVgsn (67% identity) and showed an equidistant relationship with SIVcol and SIVolc (48% identity) (data not shown).

The phylogenetic relationship between SIVschm and other primate lentiviruses was investigated further by neighbour joining (Fig. 2). Genetic divergence is illustrated by the long branch in the phylogenetic tree leading to SIVschm. However, SIVschm forms a well-supported cluster with viruses isolated from greater spot-nosed monkeys (SIVgsn) (100% bootstrap support).

The characterization of a SIV from C. ascanius schmidtii adds another virus to the growing number of immunodeficiency Fig. 2. Phylogenetic analysis of the SIVschm partial pol gene sequence. Analysis was performed with PAUP, version 4.0b10. A neighbour-joining tree was constructed with distances calculated using Kimura's two-parameter method. Bootstrap values are the result of 1000 resamplings and are given as percentages. Phylogeny was determined by comparing the pol gene sequence with other published SIV and HIV sequences. Analysis using a smaller fragment of 415 nt, but with a larger dataset, gave a similar result (data not shown).
viruses infecting African monkeys and apes. SIV infections in African monkeys are more common than was thought a few years ago and show an intriguing variety of lineages (Courgnaud et al., 2003). The picture becomes even more complicated because of the existence of mosaic viral genomes (Courgnaud et al., 2002, 2003; Georges-Courbot et al., 1998; Hu et al., 2003; Souquiere et al., 2001). The extensive variety of SIVs and the fact that natural SIV infections do not appear to cause disease in their natural host point to an ancient host–virus relationship in African nonhuman primates.

SIVgsn is particularly interesting as its genome contains a vpu gene, which was seen as a unique feature of the chimpanzee/HIV lineage. SIVgsn has a mosaic genome consisting of a gag/pol region, which is most related to that of SIVsyk (Fig. 2), and an env gene, which has the most similarity to SIVcpz env. Despite our efforts, we were not able to amplify the essential 3’ end of the SIVschm genome applying multiple sets of primers designed on the basis of published SIV sequences and lineages. The difficulty of amplifying other regions of the SIVschm genome may reflect its distinction from known SIVs. The lack of high-quality DNA (due to the poor quality of the starting material) has prevented the use of the long PCR method, which targets unintegrated circular DNA, as described by Courgnaud et al. (2002). It thus remains to be seen whether the possession of a vpu gene is a general feature of the SIVgns/SIVschm cluster and if a SIVcpz-like env is unique to SIVgns. By solving this question, we may gain additional insight into the possible chimeric origin of SIVcpz in chimpanzees and, thus, also the origin of HIV-1.

REFERENCES


