A novel simian immunodeficiency virus from black mangabey (Lophocebus aterrimus) in the Democratic Republic of Congo

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Simian immunodeficiency viruses (SIVs) naturally infect a wide range of wild African non-human primates (Hahn et al., 2000; Hayami et al., 1994; Peeters et al., 2002). On the basis of their genomic sequences, SIVs are currently classified into six major phylogenetic lineages: (i) SIVcpz in chimpanzees (Pan troglodytes), which clusters with human immunodeficiency virus type 1 (HIV-1) (Corbet et al., 2000; Gao et al., 1999; Huet et al., 1990); (ii) SIVsm in sooty mangabeys (Cercocebus atys), which clusters with HIV-2 (Chen et al., 1996; Hirsch et al., 1989); (iii) SIVagm in four species of African green monkeys (Chlorocebus aethiops) (Allan et al., 1991; Fukasawa et al., 1988); (iv) SIVamd-1 in mandrills (Mandrillus sphinx), which forms a cluster with SIVlhoest in the Hoest monkeys (Cercopithecus lhoesti) and SIVsun in sun-tailed monkeys (Cercopithecus solatus) (Beer et al., 1999; Hirsch et al., 1999; Tsujimoto et al., 1988, 1989); (v) SIVsyk in Sykes’ monkeys (Cercopithecus albogularis) (Hirsch et al., 1993); and (vi) SIVcol in guereza colobus monkeys (Colobus guereza) (Courgnaud et al., 2002). SIVmus from moustached monkeys (Cercopithecus cephus) and SIVmon from mona monkeys (Cercopithecus mona) are relatives of SIVgsn from greater spot-nosed monkeys (Cercopithecus nictitans), which carry the vpu gene in their genome (Barlow et al., 2003; Courgnaud et al., 2002, 2003a). They have been designated as a new SIV lineage, although Biotte-Ruche et al. recently suggested that the clusters of SIVgsn and SIVsyk including SIVdeb should be considered as one ancestral SIV lineage that infected monkeys of the genus Cercopithecus (Bibollet-Ruche et al., 2004; Courgnaud et al., 2003a; Verschoor et al., 2004). In addition, some novel SIV strains such as SIVtal from talapoins (Miopithecus...
talapoin), SIVwrc from western red colobus (Piliocolobus badius) and SIVolc from olive colobus monkeys (Procolobus verus) have been reported, although their sequences have only been partially characterized (Courgnaud et al., 2003b; Peeters et al., 2002).

It is now widely accepted that HIV-1 originated from SIVcpz (Gao et al., 1999; Hahn et al., 2000). Bailes et al. (2003) suggested that SIVcpz could be a recombinant virus between SIVrcm from red-capped mangabeys (Cercocebus torquatus) and SIVgsm. Furthermore, viruses of the above-mentioned six major lineages may also have complex mosaic genomes (Salemi et al., 2003). To understand better the evolutionary relationships among primate lentiviruses, a search for additional SIVs from other non-human primate species is essential. Thus, our research efforts have focused on non-human primates whose habitats are adjacent to or overlap the habitat of common chimpanzees in the forests of central Africa. In the present study, we genetically characterized a new SIV strain from a black mangabey (Lophocebus aterrimus) originally isolated in the Democratic Republic of Congo (DRC).

The animal was caught in the wild in 2001 in the Bas-Congo region, in the south-eastern part of the DRC, and kept in a separate cage for 1 year at the Kinshasa Zoo. Peripheral blood was collected in 2002 using acid citrate glucose anticoagulant solution. A plasma sample was initially tested for antibodies using a commercial particle agglutination kit (Genedia HIV-1/2, Fujirebio) and showed positive reactivity, with an antibody titre of 1:64. We then conducted a Western blot assay using commercial Western blot kits (HIV-1 and -2 LAV blot; Bio-Rad) and demonstrated strong reactivity against p18 and p25 (HIV-1 core antigens), p26 (HIV-2 core antigens) and gp105 (HIV-2 glycoprotein) (data not shown). These data suggested that this monkey was infected with an SIV strain related to HIV-1 or -2.

We carried out nested PCR to amplify the viral pol region using chromosomal DNA extracted from peripheral blood mononuclear cells. DNA was extracted using the QIAamp Blood DNA mini kit (Qiagen) according to the manufacturer’s instructions. We first amplified a fragment of 150 bp in the pol region using oligonucleotide primers DR1 (5’-TRCAYACAGGRGCWGAYGA-3’) and DR2 (5’-AIADRT-CATCCATRTAYTG-3’) for the first round and primers DR4 (5’-GGIATWCCICAYCCDGCAGG-3’) and DR5 (5’-GGIGAYCYTTCAYCCYTGHGG-3’) for the second round (Clewley et al., 1998; Courgnaud et al., 2002). Amplification was carried out with an initial denaturation at
94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 45 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 5 min. We succeeded in amplifying a 150 bp fragment from this seropositive sample. The PCR product purified from an agarose gel was subcloned into the pUC119 (Takara Bio) vector and sequenced using a cycle sequencing kit and automated sequencers (BigDye Terminator Cycle Sequencing Ready Reaction, ABI 373 and 3100; Applied Biosystems). We then performed a semi-nested PCR to amplify a 1800 bp fragment using the primers DR1 and Unipol2 (5′-CCCCTATTCCTCCCTTTTCTTTAAA-3′) for the first-round PCR and bkmpol1 (5′-GGATAGTCTTACTATTCCAG-3′) and Unipol2 for the second-round PCR (Miura et al., 1990). The primer bkmpol1 was specifically designed on the basis of the sequence of the 150 bp fragment. PCR was carried out as described above but with cycle conditions of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 3 min. The sequence of the obtained fragment was determined as described above.

The SIV derived from the black mangabey was designated SIVbkm strain CDM201. We constructed a phylogenetic tree to compare the sequence of SIVbkm CDM201 with sequences of representative isolates of SIVs and HIVs. Sequences were aligned using CLUSTAL W with minor manual modifications and a phylogenetic tree was constructed using the maximum-likelihood method with the MOLPHY program (Higgins & Sharp, 1989; Adachi & Hasegawa, 1996; Yamaguchi-Kabata & Gojobori, 2000; Yamaguchi-Kabata et al., 2004). A phylogenetic tree based on 1930 bp fragments of the pol region showed that SIVbkm was divergent from other SIV strains, but relatively close to SIVgsm and SIVsyk group isolates (Fig. 1). Subsequently, we carried out a distance plot analysis to investigate the extent of sequence differences (Takehisa et al., 1999). The reference strains used were HIV-1 groups M (strain HXB2), O (ANT70), SIVcpz (US), SIVsm (SL92L), SIVagm (TYO-1), SIVmnd-1 (GB1), SIVsyk (syk173), SIVcol (CGU-1), SIVgsm (99CM71), SIVrcm (Ngm), SIVmon (NG1) and SIVmus (01CM1085). The genetic distance (estimated using Kimura’s two-parameter method) between each selected pair of sequences was determined by moving a window of 300 bp along the genome alignment in 10 bp increments and the distance was plotted at the midpoint. SIVmon exhibited a relatively close distance to SIVbkm throughout the pol reverse transcriptase (RT) to integrase (IN) region and, interestingly, HIV-1 group O strain ANT70 showed a close relationship to SIVbkm, with the shortest distance in the RNase H region (Fig. 2). At the amino acid level, SIVbkm CDM201 showed moderate similarities with three other primate lentiviruses, SIVgsm (62 ± 5%), SIVmon (62 ± 0%) and SIVsyk (59 ± 3%) (Table 1). However, in the RNase H region, SIVbkm CDM201 showed relatively high similarities to HIV-1 group O (ANT70) (68-6 %), SIVrcm

**Fig. 2.** Distance plots comparing SIVbkm CDM201 with representative primate lentiviruses. The sequences used were HIV-1 groups M (HXB2) and O (ANT70), SIVcpzUS, SIVcm, SIVsm, SIVagm, SIVmnd-1, SIVsyk, SIVcol, SIVgsm, SIVmon and SIVmus. The genetic distance was estimated using Kimura’s two-parameter method (Kimura, 1980). The alignment was sectioned into 300 bp segments, which were moved along the genome in 10 bp increments. The distance value for each segment was plotted at the midpoint.
Also help to understand the origin and evolution of HIV-1. A better understanding of these events may lead to an understanding of the origin of the HIV/SIV lineages since the SIVgsn lineage carries the vpu gene may lead to an understanding of the origin of the vpu gene from greater spot-nosed monkeys (Cercopithecus ascanius) in Cameroon. Strongly related to the SIVgsn and SIVsyk lineages, all of which were isolated from monkeys of the genus Lophocebus. SIVbkm was found to be related to the SIVgsn and SIVsyk lineages, all of which were isolated from monkeys of the genus Cercopithecus (Barlow et al., 2003; Courgnaud et al., 2002, 2003a; Bibollet-Ruche et al., 2004). These relationships were supported by high bootstrap values. Bibollet-Ruche et al. (2004) stated that the SIVsyk and SIVgsn lineages have the same ancestor and evolved with host-species specialization. The phylogenetic relationships between SIVbkm and the SIVsyk/SIVgsn cluster suggest that cross-species transmission occurred between species of the genus Cercopithecus and a species of the genus Lophocebus. Clarifying the cross-species transmission between black mangabeys and Cercopithecus monkeys and the spread of the ancestral virus of this cluster may lead to an understanding of the origin of the vpu gene, since the SIVgsn lineage carries vpu, whereas the SIVsyk lineage does not. A better understanding of these events may also help to understand the origin and evolution of HIV-1.

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### Table 1. Percentage amino acid identity in the pol region between SIVbkm CDM201 and representatives of other HIV/SIV lineages

<table>
<thead>
<tr>
<th>HIV/SIV strains</th>
<th>Amino acid identity (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RT–IN</td>
</tr>
<tr>
<td>HXB2</td>
<td>55.7</td>
</tr>
<tr>
<td>YBF30</td>
<td>55.4</td>
</tr>
<tr>
<td>ANT70</td>
<td>56.0</td>
</tr>
<tr>
<td>SIVcpz</td>
<td>55.4</td>
</tr>
<tr>
<td>SIVrcm</td>
<td>57.4</td>
</tr>
<tr>
<td>SIVmnd-2</td>
<td>57.1</td>
</tr>
<tr>
<td>HIV-2 D205</td>
<td>53.6</td>
</tr>
<tr>
<td>SIVsm</td>
<td>55.0</td>
</tr>
<tr>
<td>SIVagm</td>
<td>55.0</td>
</tr>
<tr>
<td>SIVmnd-1</td>
<td>53.8</td>
</tr>
<tr>
<td>SIVsyk</td>
<td>59.3</td>
</tr>
<tr>
<td>SIVcol</td>
<td>46.5</td>
</tr>
<tr>
<td>SIVolc</td>
<td>47.1</td>
</tr>
<tr>
<td>SIVgsn</td>
<td>62.5</td>
</tr>
<tr>
<td>SIVmon</td>
<td>62.0</td>
</tr>
</tbody>
</table>

Numbers in bold indicate the three highest identities in each respective region.

(70.3%) and SIVmnd-2 (68.6%). In the RT and IN regions, the similarities between SIVbkm CDM201 and these three viruses were relatively low.

In the present study, we have described a novel primate lentivirus, SIVbkm, from a black mangabey in the DRC. This is the first report to characterize genetically an SIV from monkeys of the genus Lophocebus. SIVbkm was found to be related to the SIVgsn and SIVsyk lineages, all of which were isolated from monkeys of the genus Cercopithecus (Barlow et al., 2003; Courgnaud et al., 2002, 2003a; Bibollet-Ruche et al., 2004). These relationships were supported by high bootstrap values. Bibollet-Ruche et al. (2004) stated that the SIVsyk and SIVgsn lineages have the same ancestor and evolved with host-species specialization. The phylogenetic relationships between SIVbkm and the SIVsyk/SIVgsn cluster suggest that cross-species transmission occurred between species of the genus Cercopithecus and a species of the genus Lophocebus. Clarifying the cross-species transmission between black mangabeys and Cercopithecus monkeys and the spread of the ancestral virus of this cluster may lead to an understanding of the origin of the vpu gene, since the SIVgsn lineage carries vpu, whereas the SIVsyk lineage does not. A better understanding of these events may also help to understand the origin and evolution of HIV-1.

### References


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