Short Communication

Full-length genome sequence of a simian immunodeficiency virus (SIV) infecting a captive agile mangabey (*Cercocebus agilis*) is closely related to SIVrcm infecting wild red-capped mangabeys (*Cercocebus torquatus*) in Cameroon

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Simian immunodeficiency viruses (SIVs) are lentiviruses that infect an extensive number of wild African primate species. Here we describe for the first time SIV infection in a captive agile mangabey (*Cercocebus agilis*) from Cameroon. Phylogenetic analysis of the full-length genome sequence of SIVagi-00CM312 showed that this novel virus fell into the SIVrcm lineage and was most closely related to a newly characterized SIVrcm strain (SIVrcm-02CM8081) from a wild-caught red-capped mangabey (*Cercocebus torquatus*) from Cameroon. In contrast to red-capped mangabeys, no 24 bp deletion in CCR5 has been observed in the agile mangabey. Further studies on wild agile mangabeys are needed to determine whether agile and red-capped mangabeys are naturally infected with the same SIV lineage, or whether this agile mangabey became infected with an SIVrcm strain in captivity. However, our study shows that agile mangabeys are susceptible to SIV infection.

Simian immunodeficiency viruses (SIVs) are primate lentiviruses that infect an extensive number of wild African primate species. To date, serological and/or molecular evidence for SIV infection has been reported in >40 African primates and has been confirmed by viral sequence analysis in the majority of these (VandeWoude & Apetrei, 2006; Van Heuverswyn & Peeters, 2007; Locatelli *et al.*, 2008; Liégeois *et al.*, 2009; Goldberg *et al.*, 2009). The genetic diversity among non-human primate lentiviruses is extremely complex but generally each primate species is infected with a species-specific virus, which forms monophyletic lineages in phylogenetic trees. There are many examples of co-evolution between viruses and their hosts, but cross-species transmission, sometimes followed by recombination between distant SIVs, is not exceptional and one species can even harbour two different SIVs. Phylogenies for some SIVs and their hosts suggest co-evolution over long time periods, like SIVagm in the different African green monkey species (*Chlorocebus* spp.) or SIVs in arboreal *Cercopithecus* species (Bibollet-Ruche *et al.*, 2004; Wertheim & Worobey, 2007). There are also numerous examples of cross-species transmission of SIVs between primates with overlapping habitats or living in polyspecific associations. For example, SIVagm from African green monkeys has been transmitted to patas monkeys in west Africa (Bibollet-Ruche *et al.*, 1996) and to yellow and chacma baboons in South Africa (Jin *et al.*, 1994; van Rensburg *et al.*, 1998). There are also more complex examples of cross-species transmission, such as SIVs transmitted between greater spot-nosed monkeys (SIVgsn) and moustached monkeys (SIVmus), followed by recombination as seen for SIVmus-2 in moustached monkeys in Cameroon (Aghokeng *et al.*, 2007). One of the most striking examples of cross-species transmission followed by recombination is SIVcpz in chimpanzees. The 5′ region of SIVcpz is most similar to SIVrcm from red-capped mangabeys, and the 3′ region is closely related to SIVgsn from greater spot-nosed monkeys (Courgnaud *et al.*, 2002; Bailes *et al.*, 2003). It has also been shown that chimpanzees

transmitted their virus to sympatric gorillas (Van Heuverswyn et al., 2006).

Numerous cross-species transmissions among different primate species have occurred. Both human immunodeficiency virus (HIV)-1 and HIV-2 in humans are the result of cross-species transmission of SIVs from African primates (Hahn et al., 2000). The closest simian relatives of HIV-1 are SIVcpz and SIVgor, in chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla*), respectively, and which are both from west-central Africa (Keele et al., 2006; Van Heuverswyn et al., 2006). SIVsmm in sooty mangabeys (*Cercocebus atys*) from west Africa, on the other hand, are the closest relatives of HIV-2 (Santiago et al., 2005).

Despite the increasing number of SIV lineages that have been described recently, our knowledge of SIVs in their natural hosts remains limited. This is because only few viruses have been characterized for each species and for some species no, or only limited, sequence information is available. In the present study, we characterized for the first time the complete genome of an SIV infecting an agile mangabey (*Cercocebus agilis*) housed in a Cameroonian zoo. The agile mangabey (animal 703, sample no. 312) was confiscated in June 2000 by authorities of the Cameroonian Ministry of Environment and Forestry in a village 25 km away from Yaoundé, the capital city of Cameroon, where the animal was kept as a pet. The animal was subsequently transferred to the Yaoundé Zoo and, during the quarantine period, blood was drawn for the routine veterinary check-up, which included HIV/SIV serology done at the Centre Pasteur du Cameroun in Yaoundé. The agile mangabey sample showed antibodies that were cross-reactive with HIV antigens using commercial HIV screening assays, two rapid tests [Determine (Abbott) and Multispot (Sanofi Diagnostics Pasteur)] and an ELISA (Genscreen; Sanofi Diagnostics Pasteur). The sample also showed reactivity with all HIV-2 proteins, including Env proteins, on a commercial Western blot for HIV-2 (New LAV Blot II; Bio-Rad). The species origin was also confirmed by DNA analysis based on 12S rRNA gene sequencing as previously described (Van Der Kuyl et al., 2000). DNA was extracted and a 330 bp fragment in *pol* was amplified and sequenced. Phylogenetic analysis revealed that SIVagi-00CM312 was closely related to representatives from the SIVrcm lineage (Nerrienet et al., 2002). Therefore we also attempted to characterize the full-length genome of an SIVrcm strain from Cameroon because full-length SIVrcm sequences were only available from animals from Gabon and Nigeria (Georges-Courbot et al., 1998; Beer et al., 2001). Whole blood was collected, as previously described in 2002, from a red-capped mangabey (sample no. 8081) during a large survey on primate bushmeat (Aghokeng et al., 2010a) which aimed to estimate the extent of human exposure to SIVs through hunting and butchering primate bushmeat. The sample was collected in south-west Cameroon and has previously been shown to be infected with SIVrcm by detection of partial *pol* sequences.

For the two animals, the full-length SIVagi-00CM312 and SIVrcm-02CM8081 (GenBank accession numbers HM803690 and HM803689, respectively) sequences were obtained by amplification of overlapping PCR fragments. The primers used to obtain full-length SIVagi and SIVrcm sequences are provided in Supplementary Table S1 (available in JGV Online). Amplification and sequencing methods were done as previously described (Liégeois et al., 2009; Aghokeng et al., 2010b). PCR amplifications were performed using an Expand Long Template PCR kit (Roche) according to the manufacturer's instructions. Each amplification reaction program included a manual hot start at 94 °C, followed by 35 or 40 cycles with a denaturation step at 94 °C for 20 s, an annealing temperature set according to the primers' melting temperatures, an annealing time of 30–60 s and an extension time which varied according to the size of the expected fragment (1 min kbp^{-1}). PCR products were purified by gel electrophoresis (1 % agarose) and sequenced directly using cycle sequencing and dye terminator methodologies (ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq FS DNA polymerase; Applied Biosystems) on an automated sequencer (ABI 3130XL; Applied Biosystems). The full-length genome sequence was constructed by assembling overlapping sequences into contiguous ones using SeqMan II software (DNASTAR). The full-length genomes generated were 9511 and 9592 bp in length for SIVagi-00CM312 and SIVrcm-02CM8081 respectively. The new sequences were compared to those of other primate lentiviruses, and SIVagi-00CM312 and SIVrcm-02CM8081 showed the expected reading frames for *gag, pol, vif, vpr, tat, rev, env* and *nef*. Both also contained a *vpx* gene, which is characteristic of the HIV-2/SIVsmm and SIVrcm lineages. For both viruses, the LTRs contained all the characteristic features of other primate lentiviruses.

Nucleotide and protein sequences were aligned using MEGA 4 and CLUSTAL X version 1.8 (Thompson et al., 1997; Kumar et al., 2004), with minor manual adjustments. Sites that could not be unambiguously aligned were excluded. Proteome sequences were generated by joining deduced Gag, Pol, Env and Nef amino acid sequences. The carboxy-terminal Gag and Env amino acid sequences that overlapped with Pol and Nef amino acid sequences, respectively, were excluded. The predicted protein sequences encoded by SIVrcm-02CM8081 and SIVagi-00CM312 were compared to representatives of known HIV/SIV lineages. In order to study whether the newly characterized SIVrcm and SIVagi sequences were recombinant with any of the other SIV lineages, similarity plot analysis was performed with SIMPLOT version 2.5 (Lole et al., 1999) using a sliding window of 200 aa moved in steps of 20 aa. Similarity plot analysis was performed on the concatenated amino acid sequence alignment and the following strains were used in the analysis: SIVtalCM8023, SIVdebCM40, SIVgsnCM166, SIVmonCML1, SIVmusCM1085, SIVden, SIVrcmNgm, SIVrcmGAB1, SIVrcm8081, SIVagi312, SIVmnd14cg, SIVsyk173, SIVlho, SIVsunL14, SIVmndGB1, SIVwrc0498, SIVsmm251, SIVagmVER155, SIVcpzUS, SIVcolCGU1,

SIVolc97CI12. The similarity plot analysis showed that SIVagi-00CM312 is most closely related to the SIVrcm lineage over the entire genome (Fig. 1). Overall, the amino acid similarities between SIVagi and representatives of the SIVrcm lineage are between 80 and 94 %, which are lower than the similarities observed between the different SIV lineages and are similar to those generally observed among SIV strains from the same SIV lineage (Aghokeng et al., 2007, 2010b).

We constructed evolutionary trees from Gag, Pol and Env amino acid sequences to estimate the relatedness of the new SIVagi-00CM312 strain to other primate lentiviruses in these three main genomic domains. Phylogenies were inferred by the Bayesian method (Yang & Rannala, 1997) which was implemented in MRBAYES version 3.1 (Huelsenbeck & Ronquist, 2001). Markov chain Monte Carlo analyses were run for 1.5, 3 and 3 million generations, and sampled every 100 generations, for Gag, Pol and Env, respectively, with a 10 % burn-in. The mixed model in MRBAYES indicated that the rtRET model of amino acid change (Dimmic et al., 2002) was the most appropriate; this model was thus used with gamma distribution rates across sites. Parameters were examined with the Tracer program (http://tree.bio.ed.ac. uk/software/tracer/). In all three regions, the novel SIV$_{agi}$-00CM312 strain clustered within the SIVrcm lineage, which includes the previously reported SIVrcm strains from Nigeria and Gabon, SIVrcmNgm and SIVrcmGAB1, respectively, and the novel SIVrcm-02CM8081 strain from Cameroon (Fig. 1). Interestingly, the SIVagi-00CM312 strain seems to be more closely related to the SIVrcm-02CM8081 strain from Cameroon, confirming the observations from the similarity plot analysis (Fig. 1).

It has been shown previously that SIVrcm uses CCR2b as a co-receptor for viral entry because of a 24 bp deletion in the CCR5 gene that rendered this co-receptor non-functional (Chen et al., 1998). To check whether agile mangabey 312 and red-capped mangabey 8081 present undeleted CCR5, we sequenced the portion of interest of this gene. We amplified the CCR5 region using PCR primers that hybridized to a CCR5 region flanking the 24 bp deletion as previously described (Chen et al., 1998). The amplification programme was 94 °C for 2 min, followed by 35 cycles of 95 °C for 15 s, 55 °C for 20 s and 72 °C for 20 s, and a final extension of 72 °C for 10 min. Using this primer pair, 189 or 213 bp CCR5-specific fragments with or without the 24 bp deletion were amplified from genomic DNA. We found that, in contrast to all red-capped mangabeys, in agile mangabey 312 the 24 bp section of CCR5 was not deleted (data not shown).

Previous studies have shown that SIVrcm infection is frequent, with prevalences that can reach up to 50 % among wild red-capped mangabeys (Aghokeng et al., 2010a). Moreover, SIVrcm infection also seems to be widespread throughout the geographical range of red-capped mangabeys, between the coastal areas of east Nigeria (Cross River) and southern Gabon (Georges-Courbot et al., 1998; Beer

et al., 2001). In contrast, samples from wild agile mangabeys were obtained during a previous survey on SIV infection in primate bushmeat at different sites across Cameroon. Among a total of 182 samples from agile mangabeys no single SIV-infected animal could be identified, thus suggesting either the absence or low prevalence of SIV infection in this monkey species (Aghokeng et al., 2010a). In contrast to red-capped mangabeys, the geographical range of agile mangabeys is significantly larger and covers a large area across central Africa above the Congo river ranging from western Cameroon to the eastern part of the Democratic Republic of Congo. Both species live in swamp forest but they are allopatric and there is only a small area in Cameroon (close to Meyo/Sangmelina) where both species could overlap (Kingdon, 1997; Gautier-Hion et al., 1999; Groves, 2001). From the sequence and phylogenetic tree analysis, it is clear that the virus obtained from the agile mangabey in our study belongs to the SIVrcm lineage. Moreover the novel SIVagi strain seems to be even more closely related to the SIVrcm strains from red-capped mangabeys from Cameroon than to those from the surrounding countries. The SIVagi strain described here is thus most probably the result of a cross-species transmission. As mentioned above, the strain was derived from a captive animal that was kept as a pet in a hotel for at least 3 years together with other monkeys, although they did not share the same cage. The other captive monkeys (an olive baboon and a mandrill) kept in this hotel were also confiscated by the Cameroonian Ministry of Environment and screened for SIV infection but none of them were shown to be SIV positive. However, we do not know whether other primates were previously housed in this hotel and it cannot be entirely excluded that the animal has never been in contact with SIV-infected primates. There is only a small region in Cameroon where the geographical range of both species overlaps and both species share similar habitats, i.e. swamp forest (Gautier-Hion et al., 1999). It is thus also possible that the animal tested had been infected naturally through contact with red-capped mangabeys. However, the precise place where the animal was captured in the wild is not known. Except for a small geographical area, agile and red-capped mangabeys are currently non-sympatric, but it is not unlikely that the situation was different in the past and that agile and red-capped mangabeys are naturally infected with closely related SIVs from a common ancestor. However, the phylogenetic data and low prevalence suggest a more recent transmission. SIVrcm has the unusual property of utilizing CCR2B rather than CCR5 as its primary co-receptor for viral entry (Chen et al., 1998). Genetic analysis of CCR5 alleles of the captive mangabey revealed the absence of this 24 bp deletion in this monkey, suggesting that the SIVagi-00CM321 strain uses CCR5 as its primary co-receptor. However, it cannot be excluded that CCR2B is also used by SIVagi-00CM321.

This study confirmed for the first time the existence of SIV infection in agile mangabeys in Cameroon. Unfortunately, our study does not allow us to conclude whether agile and
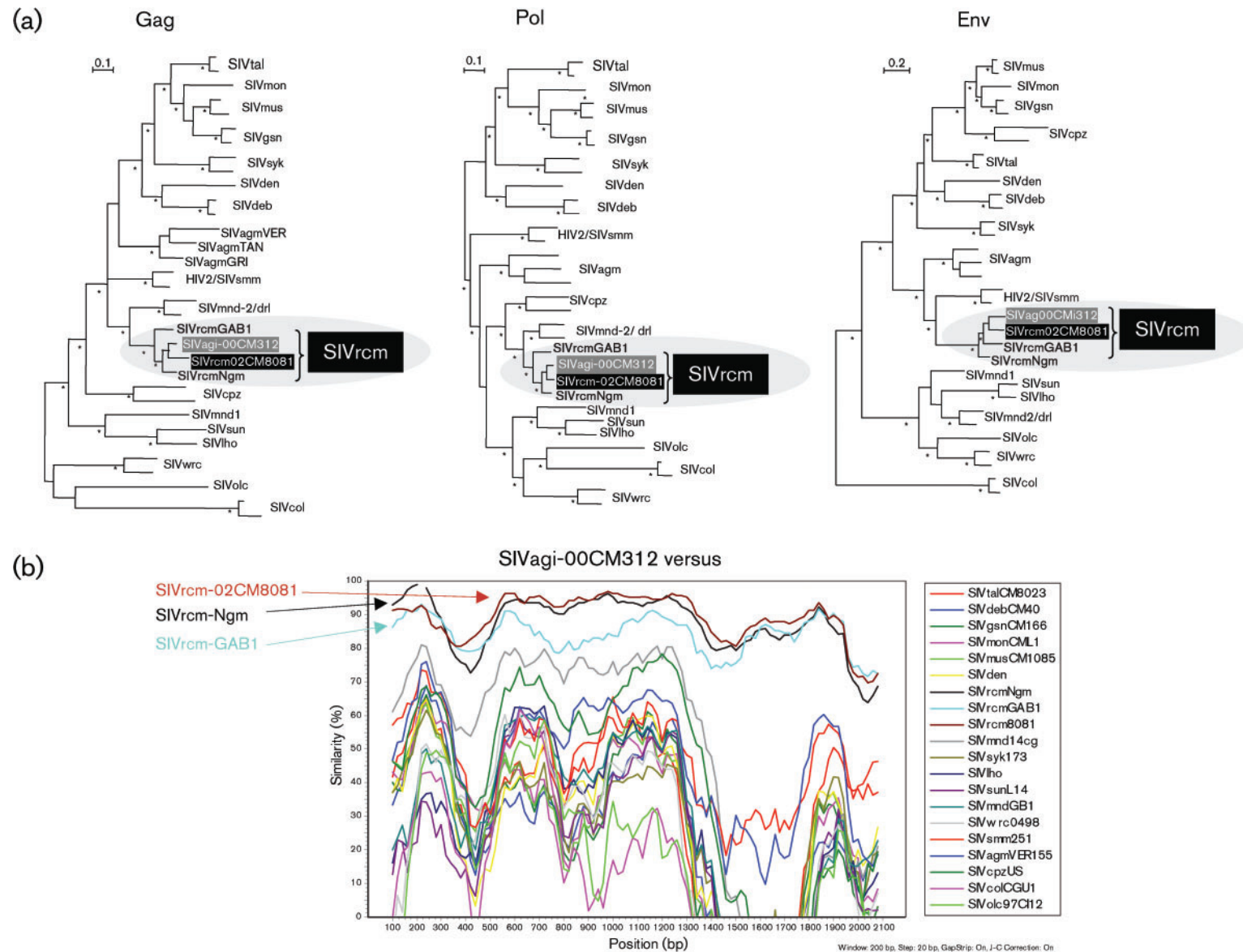
Fig. 1. (a) Phylogenetic relationship of the newly derived SIVagi-00CM312 strain (in grey box) to previously described SIVrcm viruses (bold), the newly obtained SIVrcm virus (black box) and other SIV lineages in Gag, Pol and Env regions. The maximum-likelihood trees were inferred from protein sequence alignments. *, indicates values for the estimated posterior probabilities from the Bayesian method of ⩾95 %. Bars, substitutions per site. (b) Diversity plots of concatenated protein sequences representing the extent of the genetic difference between SIVagi-00CM312 and other primate lentiviruses including the newly described SIVrcm-02CM8081 strain from Cameroon. The *y*-axis indicates the distance between the viral proteins.

red-capped mangabeys are naturally infected with the same SIV lineage, or whether this agile mangabey became infected with an SIVrcm strain in captivity. However, our study shows that agile mangabeys are susceptible to SIV infection and apparently no pathogenicity seems to be associated with this SIV infection because the animal was still in a good healthy condition 9 years after its capture. Thus, more studies on wild red-capped and agile mangabeys are necessary to clarify whether agile mangabeys are naturally infected with an SIV, and these studies should also focus on the area where the two species could overlap.

Acknowledgements

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