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Prevalence and genetic diversity of simian immunodeficiency virus infection in wild-living red colobus monkeys (*Piliocolobus badius badius*) from the Taï forest, Côte d'Ivoire

SIVwrc in wild-living western red colobus monkeys☆

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Abstract

Numerous African primates are infected with simian immunodeficiency viruses (SIVs). It is now well established that the clade of SIVs infecting west-central African chimpanzees (*Pan troglodytes troglodytes*) and western gorillas (*Gorilla gorilla gorilla*) represent the progenitors of human immunodeficiency virus type 1 (HIV-1), whereas HIV-2 results from different cross-species transmissions of SIVsmm from sooty mangabeys (*Cercocebus atys atys*).

We present here the first molecular epidemiological survey of simian immunodeficiency virus (SIVwrc) in wild-living western red colobus monkeys (*Piliocolobus badius badius*) which are frequently hunted by the human population and represent a favourite prey of western chimpanzees (*Pan troglodytes verus*). We collected faecal samples ($n = 88$) and we assessed individual discrimination by microsatellite analyses and visual observation. We tested the inferred 53 adult individuals belonging to two neighbouring habituated groups for presence of SIVwrc infection by viral RNA (vRNA) detection. We amplified viral polymerase (*pol*) (650 bp) and/or envelope (*env*) (570 bp) sequences in 14 individuals, resulting in a minimal prevalence of 26% among the individuals sampled, possibly reaching 50% when considering the relatively low sensitivity of viral RNA detection in faecal samples. With a few exceptions, phylogenetic analysis of *pol* and *env* sequences revealed a low degree of intragroup genetic diversity and a general viral clustering related to the social group of origin. However, we found a higher intergroup diversity. Behavioural and demographic data collected previously from these communities indicate that red colobus monkeys live in promiscuous multi-male societies, where females leave their natal group at the sub-adult stage of their lives and where extra-group copulations or male immigration have been rarely observed. The phylogenetic data we obtained seem to reflect these behavioural characteristics. Overall, our results indicate that wild-living red colobus represent a substantial reservoir of SIVwrc. Moreover, because of their frequent association with other monkey species, the predation pressure exerted by chimpanzees

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☆ Nucleotide sequence data reported in this paper are available in the GenBank, EMBL and DDBJ databases under the accession numbers listed in parentheses: for the pol gene: SIVwrc-04CI-196 (AM743109), SIVwrc-04CI-116 (AM743110), SIVwrc-04CI-299 (AM743111), SIVwrc-04CI-110 (AM743112), SIVwrc-04CI-115 (AM743113), SIVwrc-04CI-112 (AM743114), SIVwrc-04CI-223 (AM743115), SIVwrc-04CI-52 (AM743116), SIVwrc-04CI-237 (AM743117), for the env gene: SIVwrc-04CI-268 (AM743118) SIVwrc-04CI-52 (AM743119), SIVwrc-04CI-116 (AM743120), SIVwrc-04CI-110 (AM743121), SIVwrc-04CI-237 (AM743122), SIVwrc-04CI-175 (AM743123), SIVwrc-04CI-296 (AM743124), SIVwrc-04CI-223 (AM743125), SIVwrc-04CI-299 (AM743126), SIVwrc-04CI-280 (AM743127), SIVwrc-04CI-32 (AM743128), SIVwrc-04CI-196 (AM743129), SIVwrc-04CI-112 (AM743130).

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(*Pan troglodytes verus*) and by poachers around and inside the park, simian to simian and simian to human SIVwrc cross-species transmission cannot be excluded.

Keywords: SIV; HIV; Red colobus; Faecal samples; Microsatellites; Non-invasive sample collection; Côte d'Ivoire; Polyspecific associations; Bushmeat; Taï forest

1. Introduction

Serological evidence for simian immunodeficiency virus (SIV) infection has been identified, to date, in 39 different nonhuman primate (NHP) species in sub-Saharan Africa. SIV infection has been molecularly confirmed in 32 NHP species and in 19, full length SIV sequences were obtained (van de Woude and Apetrei, 2006). High genetic diversity is observed among the known SIVs but, generally, each primate species is infected with a species-specific virus that forms monophyletic lineages in phylogenetic trees (Bibollet-Ruche et al., 2004; Courgnaud et al., 2001). In addition, several "major SIV lineages" have been identified which represent groups of SIVs from different primate species that are more closely related to one another than they are to other SIVs. For some of these SIV lineages, virus and host phylogenies seem to match, suggesting virus/host co-speciation, but there are also numerous examples of cross-species transmissions and recombination (Bailes et al., 2003; Beer et al., 1999; Bibollet-Ruche et al., 1997; Bibollet-Ruche et al., 1996; Hirsch et al., 1999; Hu et al., 2003; Jin et al., 1994a, 1994b; Salemi et al., 2003; van Rensburg et al., 1998). Interestingly, it has also been shown that one primate species can be infected with two different SIVs (Aghokeng et al., 2007; Souquiere et al., 2001). One of the most striking examples of cross-species transmission, followed by recombination, is SIVcpz in chimpanzees from central Africa (*Pan troglodytes troglodytes*), with its $5'$ end being closest to that of SIVrcm from red capped mangabeys and the $3'$ end most closely related to those of the SIVgsn/SIVmus/SIVmon lineage from greater spot-nosed, mustached and mona monkeys (Bailes et al., 2003; Beer et al., 2001; Courgnaud et al., 2002; Santiago et al., 2002). Apparently, chimpanzees acquired their SIV infection through hunting other NHP species (Mitani and Watts, 1999; Sharp et al., 2005). With the exception of SIVcpz from chimpanzees and SIVgor from gorillas, all SIVs identified so far originate from primates belonging to the family *Cercopithecidae*, or Old World monkeys, which is subdivided into two subfamilies: *Colobinae* and *Cercopithecinae* (Disotell, 1996). The colobinae subfamily comprises three genera, *Colobus*, *Procolobus* and *Piliocolobus* (Groves, 2001). The first primate lentivirus identified in the *Colobinae* subfamily, SIVcol from mantled guerezas (*Colobus guereza*) in Cameroon, forms a separate divergent lineage in the phylogenetic tree analysis (Courgnaud et al., 2001). Subsequently, SIVwrc and SIVolc were identified in western red (*Piliocolobus badius badius*) and in olive colobus (*Procolobus verus*), respectively, in Taï National Park, Côte d'Ivoire. Phylogenetic analyses of a 2000 bp fragment in the *pol* region showed that SIVwrc and SIVolc sequences each formed species-specific monophyletic lineages but were not at all related to the SIVcol strain obtained from a mantled guereza in Cameroon (Courgnaud et al., 2003).

We know today that handling of infected NHP carcasses exposes the human population to a risk of transmission of different viruses, including SIV (Peeters et al., 2002). Today, apart from SIVsmm in sooty mangabeys (*Cercocebus atys atys*), which is recognised as the progenitor of human immunodeficiency virus type 2 (HIV-2) (Chen et al., 1995; Clavel et al., 1986), we know of SIVcpz and SIVgor from chimpanzees (*Pan troglodytes troglodytes*) from western gorillas (*Gorilla gorilla gorilla*) inhabiting west-central Africa to have given rise to human immunodeficiency virus type 1 (HIV-1), group M, N and O (Gao et al., 1999; Keele et al., 2006; Sharp et al., 2005; Van Heuverswyn et al., 2006). Moreover, it has been shown that SIVs isolated in sooty mangabeys from the Taï forest are closely related to certain HIV-2 variants playing a role in the west African HIV-2 epidemic (Santiago et al., 2005). The Taï forest is home to nine diurnal primate species, including chimpanzees, western red colobus, olive colobus, western black and white colobus, different species of guenons (diana monkeys, campbell monkeys, lesser spot-nosed and greater spot-nosed monkeys) and sooty mangabeys (McGraw et al., 2007). The red colobus (*Piliocolobus badius badius*) and the diana monkey (*Cercopithecus diana diana*) are the primate species best represented in Taï National Park, but, after the antelopes, they also are the most extensively hunted by the human population (Refisch and Koné, 2005). In addition, chimpanzees (*Pan troglodytes verus*) of the Taï forests are also known to hunt western red colobus monkeys frequently (Boesch and Boesch-Achermann, 1989). More than 1400 chimpanzees issued from U.S. research centers and zoos (18.16% were African born) have been tested for HIV cross-reactive antibodies, yet no SIV infection has been identified in the chimpanzee subspecies *P.t.verus* (Switzer et al., 2005). However, in analogy to the origin of SIVcpz in chimpanzees from central Africa, it cannot be excluded that western chimpanzees became infected with a SIV harboured by their prey. It is important to note that these animals have been screened with HIV assays, which may not be sensitive enough to detect infections with divergent SIVs. Moreover, another retrovirus, the simian T-cell leukaemia virus type 1 (STLV-1), is already circulating in both western red colobus and chimpanzees in the Taï forest (Leendertz et al., 2004).

In order to investigate the likelihood of west African chimpanzees becoming infected with SIVwrc from western red colobus monkeys and to estimate the risk of SIVwrc transmission into the human population, it is important to gain more information on SIV infection in this primate species in its natural habitat. The Taï National Park provided a unique