Overview of Primate Lentiviruses and Their Evolution in Non-human Primates in Africa

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INTRODUCTION

Simian immunodeficiency viruses (SIV) and the closely related human immunodeficiency viruses (HIV-1 and HIV-2) belong to the lentivirus subfamily of retroviruses. SIVs are a large group of viruses that are found naturally in many African primate species, and serological and/or molecular evidences for SIV infection have been reported in at least 30 African non human primates (NHP) (44, 71). Although these NHP lentiviruses are called immunodeficiency viruses, they do not induce an AIDS-like disease in their natural hosts, suggesting that they have been associated and evolved with their hosts over an extended period of time. Phylogenetic studies of primate lentiviruses provide evidence that some SIV lineages have co-evolved with their hosts, but on the other hand they show also multiple examples of cross-species transmission from simians to humans and between different simian species (44, 86).

In some cases, if cross-species transmission occurs, the virus may be pathogenic for the new host. The most striking examples of cross-species transmissions are HIV-1 and HIV-2, the etiologic agents for AIDS in humans (8, 21). Their closest simian relatives are SIVcpz in chimpanzees (*Pan troglodytes troglodytes*) from West central Africa and SIVsm in sooty mangabeys (*Cercocebus atys*) from West Africa (39, 40, 48), respectively. AIDS was first recognized in the early 1980s and now is one of the most important infectious diseases. Today more than 40 million individuals are estimated to live with HIV infection or AIDS (98). HIV-1 has spread to most parts of the world, while HIV-2 has remained largely restricted to West Africa (84). As recently shown, humans are still exposed to a plethora of primate lentiviruses through hunting and handling of primate bushmeat in central Africa (71). The possibility of additional zoonotic transfers of primate lentiviruses from species other than chimpanzees and sooty mangabeys has thus to be considered. The current HIV-1 epidemic provides evidence for the extraordinary impact that can result from such primate lentiviral zoonotic transmission events. It will thus be important to obtain a complete and accurate assessment of all SIV infected non-human primate species.

In this paper we will summarize the actual knowledge on SIV infected primates and the characteristics of the currently identified SIVs.

SIV INFECTED PRIMATES.

Natural SIV infection has only been identified in African primate species. With the exception of SIVcpz from chimpanzees, all SIVs identified originate from primates belonging to the family of *Cercopithecidae*, or Old World monkeys. *Cercopithecidae* are subdivided into two distinct subfamilies: *Colobinae* and *Cercopithecinae*. Within the *Colobinae*, the living African colobids are further represented by 3 genera, *Colobus* or black and white colobus, *Piliocolobus* or red colobus and *Procolobus* or olive colobus. The *Cercopithecinae* subfamily comprises all the non-*Colobinae* African monkeys and is further subdivided into two main tribes: *Papionini* or baboon-macaques and *Cercopithecini* or guenons. The *Papionini* tribe comprise representatives of baboons (*Papio sp*), mandrills and drills (*Mandrillus sp*), drill-mangabeys (*Cercocebus sp*) and baboon-mangabeys (*Lophocebus sp*). The *Cercopithecini* tribe includes African green monkeys (*Chlorocebus sp*), talapoins (*Miopithecus sp*), patas monkeys (*Erythrocebus sp*), Allan's swamp monkeys (*Allenopithecus sp*) and a large variety of *Cercopithecus* species (43).

Table 1 summarizes all the African primate species in which serological and/or molecular evidences of SIV have been described. Serological evidence has been described in at least 33 different species. All major SIV lineages known to date were discovered because their primate hosts had antibodies that cross-reacted with HIV-1 or HIV-2 antigens (10, 11, 15, 19, 22, 26, 33, 30, 41, 45, 72, 74, 95). The extent of this cross-reactivity is not known, and screening for SIV infection using HIV tests can thus underestimate SIV infection, but can also represent non-specific antibody binding (false positivity). PCR followed by sequence and phylogenetic analysis is therefore necessary to confirm SIV infection. Today, SIV infection was confirmed by partial or full-length genome sequencing in 27 species. The corresponding species-specific SIVs are indicated in Table 1 and are identified by a lower case letter code which in general corresponds to the initial letters of the common species name ; e.g., SIVsm for sooty mangabeys, SIVagm for African green monkeys, SIVcpz for chimpanzees, etc. Although several highly cross-reactive primer pairs have been described that can amplify sequences from a variety of divergent HIV and SIV strains, it is more than likely that only a subset of viral SIV sequences are detected, which could explain why for some primate species only serological evidence for SIV infection is actually available (22, 26). With a high probability more African primates than shown in Table 1 are carriers of an SIV, since for some species only a limited number of individuals have been tested and some have not been screened at all (99).

Genomic organization

The common structure for primate lentiviruses is LTR-*gag-pol-vif-vpr-tat-rev-env-nef*-LTR, but some lentiviruses, have an additional gene, *vpu* or *vpx* in the central part of the genome (Figure 1). The basic genome structure applies to the members of the SIVagm, SIVsyk, SIVlhoest, SIVmnd-1 and SIVcol lineages (5, 10, 26, 35, 37, 45, 46, 52, 90). SIVsm-related viruses, including HIV-2 and SIVmac, as well as SIVrcm, and SIVmnd-2 have a *vpx* gene upstream of the vpr gene (11, 17, 21, 48, 91). The deduced Vpx protein shares sequence similarity with Vpr, and therefore could have been arisen through gene duplication (94), although phylogenetic analyses suggest that *vpx* is more likely to have been acquired by nonhomologous recombination between different SIVs (87). Until recently, only the viruses belonging to the SIVcpz/HIV-1 lineage carried an additional *vpu* gene upstream of *env* (50, 101). The presence of a *vpu* gene has now also been documented in SIVs from several *Cercopithecus* species: in SIVgsn from a greater spot-nosed monkey (*C.nictitans*) in Cameroon (27), in SIVmon and SIVmus from mona

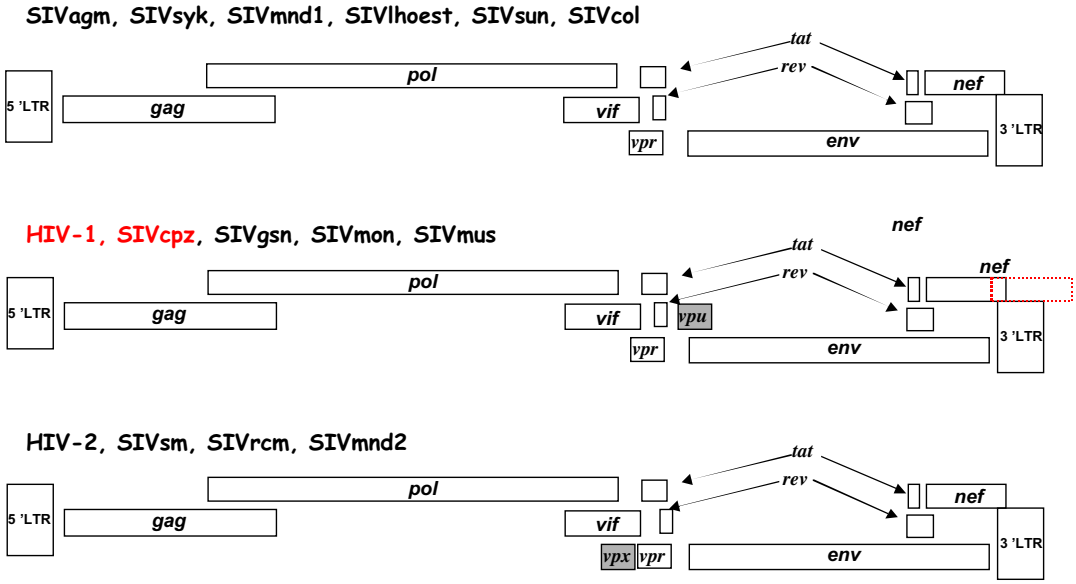

Figure 1: Genomic organization of the primate lentiviruses. In HIV-1 and SIVcpz the env and nef gene do not overlap, but in SIVgsn, mon, and mus env and nef genes overlap.

Table 1: The African non-human primates with serological and/or molecular evidence for SIV infection.

Family	*Subfamily*	*Tribe*	*Genus*	*Superspecies*	*species*	common name	SIV	geographic distribution in Africa	references
Hominidae									
	Homininae		*Pan*		*troglodytes*	common chimpanzee	SIVcpz	West to East: Senegal to Tanzania	23,39,50,80,101
Cercopithecidae									
	Colobinae		*Colobus*		*guereza*	mantled guereza	SIVcol	Central: Nigeria to Ethiopia/Tanzania	26
			Piliocolobus		*badius*	western red colobus	SIVwrc*	West: Senegal to Ghana	25
			Procolobus		*verus*	olive colobus	SIVolc*	West: Sierra-Leone to Ghana	25
	Cercopithecinae								
		Papionini	*Lophocebus*		*albigena*	gray-cheeked managabey	?	Central: Nigeria to Uganda/Burundi	71
			Papio		*anubis*	olive baboon	?	West to East: Mali to Ethiopia	71
					cynocephalus	yellow baboon	SIVagm-Ver*	Central: Angola to Tanzania	53
					ursinus	chacma baboon	SIVagm-Ver*	South: southern Angola to Zambia	100
			Cercocebus		*atys*	sooty mangabey	SIVsm	West: Senegal to Ghana	19,48
					torquatus	red-capped mangabey	SIVrcm	West Central: Nigeria,Cameroon, Gabon	11
					agilis	agile mangabey	SIVagi*	Central: Northeast Gabon to Northeast Congo	65
			Mandrillus		*sphinx*	mandrill	SIVmnd-1, SIVmnd-2	West Central: Cameroon (south of Sanaga) to Gabon, Congo	91,96
					leucophaeus	drill	SIVdrl*	West Central: southeast Nigeria to Cameroon (North of Sanaga)	22
		Cercopithecini							
			Allenopithecus		*nigrovidis*	Allen's swamp monkey	?	Central: Congo	62
			Miopithecus		*talapoin*	Angolan talapoin	SIVtal*	West Central: East coast of Angola into Congo-Zaire	69
					ogouensis	Gabon talapoin	SIVtal*	West Central: Cameroon (south of Sanaga)-Gabon	71
			Erythrocebus		*patas*	patas monkey	SIVagm-sab*	West to East: Senegal to Ethiopia, Tanzania	15
			Chlorocebus		*sabaeus*	green monkey	SIVagm-Sab	West: Senegal to Volta river	52
					aethiops	grivet	SIVagm-Gri	East: Sudan, Erithrea, Ethiopia	35
					tantalus	tantalus monkey	SIVagm-Tan	Central: Ghana to Uganda	44,90
					pygerythrus	vervet monkey	SIVagm-Ver	South: South Africa to Somalia and Angola	37
			Cercopithecus						
				diana	*diana*	Diana monkey	?	West: Sierra-Leone to Ivory Coast	62
				mitis	*nictitans*	greater spot-nosed monkey	SIVgsn	Central: forest blocks from West Africa to Congo-Zaire	27
					mitis	blue monkey	SIVblu*	East Central: East Congo to Rift-valley	13
					albogularis	sykesmonkey	SIVsyk	East: Somalia to Eastern Cape	46
				mona	*mona*	mona monkey	SIVmon*	West: Niger delta to Cameroon (north of Sanaga)	71
					pogonias	crested mona	?	West Central: Cross-river in Nigeria to Congo (east)	71
					wolfi	Wolf's mona	SIVwol*	Central: south of Congo river	32
				cephus	*cephus*	mustached guenon	SIVmus*	West central: Cameroon (south of Sanaga) to east of Congo river	71
					ascanius	red-tailed monkey	SIVasc*	Central: South-East Congo to West Tanzania	82
				lhoest	*lhoest*	l'Hoest monkey	SIVlhoest	Central: Eastern Congo-Zaire to western Uganda	9,45
					solatus	sun-tailed monkey	SIVsun	West central: tropical forest of Gabon	10
				hamlyni	*hamlyni*	owl-faced monkey	?	Central: eastern Congo-Zaire to Ruanda	66
				neglectus	*neglectus*	de Brazza's monkey	SIVdeb*	Central: Angola, Cameroon, Gabon to Uganda, western Kenya	71

** only partial sequences are available, ? only serological evidence*

(*C.mona*) and mustached (*C.cephus*) monkeys respectively, and SIVwol from a Wolf's monkey (*C.wolfi*) from the Democratic Republic of Congo (24, 32). The divergence among Vpu proteins is extremely high, less than 30% identity (27). The origin of *vpu* has not been elucidated. The only SIVs actually known to have been transmitted to humans, carried either a *vpx* or a *vpu* gene.

PHYLOGENY OF PRIMATE LENTIVIRUSES

Based on comparisons of their sequences and the functional similarity of their genes, primate lentiviruses, for which full-length genome sequences are available, were classified into 6 approximately equidistant lineages. These lineages have been identified and named according to the chronological order of their discovery and the genetic characterization of the different SIVs. They are represented by:

- SIVsm from sooty mangabeys (*Cercocebus atys*) together with HIV-2
- SIVcpz from chimpanzees (*Pan troglodytes*) together with HIV-1
- SIVagm from four species of African green monkeys (members of the *Chlorocebus* genus)
- SIVsyk from Sykes monkeys (*Cercopithecus albogularis*)
- SIVlhoest from l'Hoest monkeys (*Cercopithecus lhoesti*) and SIVsun from sun-tailed monkeys (*Cercopithecus solatus*) together with SIVmnd-1 from a mandrill (*Mandrillus sphinx*).
- SIVcol from a colobus monkey (*Colobus guereza*).

As more and more SIVs have been fully characterized, it was soon realized that some viruses appear to have a mosaic genome structure. The first mosaic SIV was SIVagmSab, isolated from a West African sabaeus monkey (52). More recently, characterization and phylogenetic analysis of full-length genome sequences of SIVrcm from red-capped mangabeys, SIVmnd2 from mandrills in Cameroon, and SIVgsn from greater spot-nosed monkeys in Cameroon, showed that these SIVs present also discordant phylogenies depending on the region of their genome studied (11, 27, 91, 93). This means that recombination events have occurred between viruses in the wild, and indicates that both cross-species transmission and coinfection with highly divergent viral strains are possible.

SIVs from other African primates have been partially characterized, mainly in the *pol* gene (see table 1). They may represent additional distinct lineages but only analysis of the complete genome will allow to establish the exact phylogenetic relationship between these SIVs and other primate lentiviruses.

Figure 2 represents phylogenetic trees in Pol (2a) and Env (2b), showing the 6 initially described HIV/SIV lineages and the lineages with discordant phylogenies for which full-length sequences are available.

Below we will describe more in detail the discovery, and characteristics of the different SIVs, for which the complete genome and partial sequences are available.

FULL-LENGTH GENOME CHARACTERIZATION OF SIVs

The SIVsm/HIV-2 lineage

The macaque viruses were among the first SIV strains to be characterized (29, 59). SIVmac strains have been isolated from captive macaque species with AIDS-like symptoms but macaques in the wild in Asia appeared not to be infected with SIV (12, 56, 104). Soon after the discovery of SIVmac between 1983 and 1985, HIV-2 was isolated from patients with AIDS originating from West Africa and SIVsm was isolated from healthy captive sooty mangabeys (*Cercocebus atys*) (7, 21, 62). Molecular analyses revealed that HIV-2 and SIVsm were closely related to each other and to SIVs from macaques (48). The absence of SIV infection of macaques in the wild and the fact that they develop an AIDS like disease when they are infected with SIV, suggest that SIVmac resulted from transmissions of SIV from sooty mangabeys to macaques in captivity. On the other hand, the subsequent isolation and characterization of SIVsm strains from free-ranging and pet sooty mangabeys in their natural habitat in West Africa (Guinea-Bissau to Côte d'Ivoire) clearly confirmed that mangabeys are the natural host for SIVsm (19, 20, 75). SIVsm prevalences can reach up to 20% in the wild.

The close phylogenetic relationship and the similarities in viral genome organization indicate that

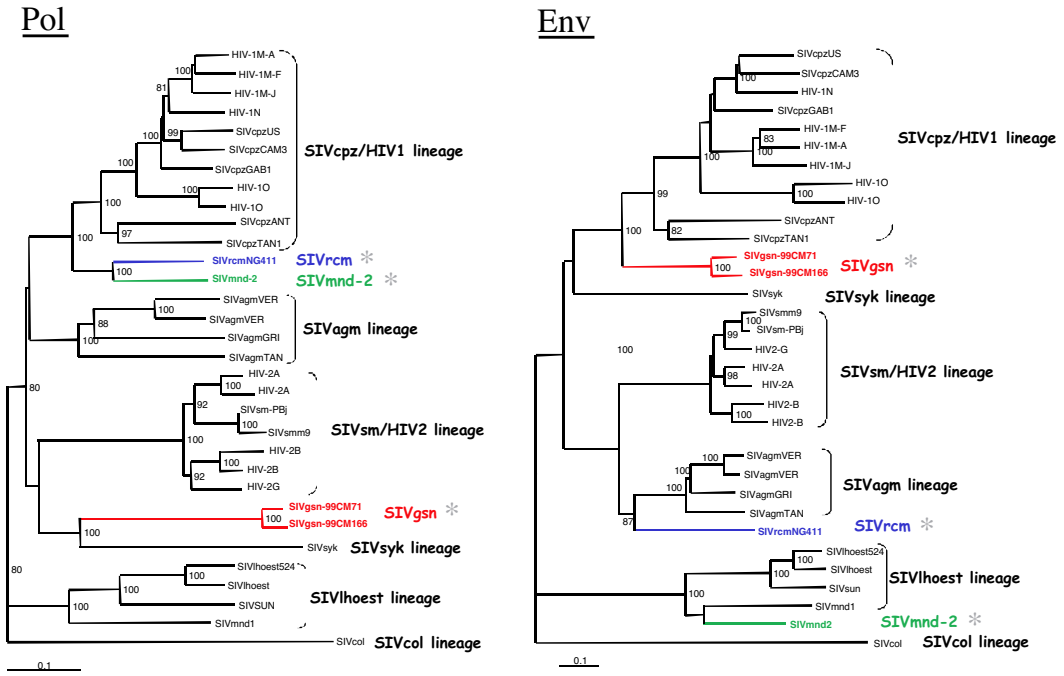

*Figure 2: Evolutionary relationships among the different primate lentivurses, for which full length genome sequences are available. Phylogenetic trees were derived by neighor-joining analysis of Pol (2a) and Env (2b) amino acid sequences. The 6 major SIV lineages are in black, and the recently described SIVs with discordant phylogenies are in gray (marked by *). Branch lengths are drawn to scale (the bar indicates 10% divergence) and only bootstrap values above 80% are shown.*

HIV-2 is the result from a zoonotic transmission from SIVsm from sooty mangabeys to humans in West Africa. The natural habitat of sooty mangabeys coincides with the geographical region where HIV-2 is prevalent in West Africa and sooty mangabeys are regularly hunted for food or kept as pets, allowing thus direct contact between mangabeys and humans (63). More detailed phylogenetic analysis showed even that cross-species transmissions from SIVsm to humans occurred on several occasions (44). Several of the HIV-2 subtypes have only been found in countries where sooty mangabeys are largely present. Seven subtypes (A - G) of HIV-2 have been described so far (105). Only subtypes A and B are largely represented in the HIV-2 epidemic, with subtype A in the western part of West Africa (Senegal, Guinea-Bissau) and subtype B being predominant in Ivory Coast (28, 34, 77, 83). The other subtypes have been documented in one or few individuals only. Except for subtype G, which was isolated from a blood donor in Ivory Coast (105), subtypes C, D, E and F were isolated in rural areas in Sierra Leone and Liberia and these viruses are more closely related to the SIVsm strains obtained from sooty mangabeys found in the same area than to any other HIV-2 strains (18, 20). Consequently, HIV-2 and SIVsm lineages are phylogenetically interspersed. This suggests that the different clades of HIV-2 must be the result of multiple independent cross-species transmissions of SIVsm into the human population(44). Recently, a remarkable genetic diversity among SIVsm strains has been shown, 4 new SIVsm lineages were identified in captive sooty mangabeys which were infected with SIVsm before their arrival in a colony in the United States (61).

The SIVcpz/HIV-1 lineage

SIVcpz, isolated from chimpanzees (*Pan troglodytes*), is closely related to HIV-1. Actually, 8 SIVcpz strains are described: 2 from Gabon (SIVcpz-gab1 and gab2), 3 from Cameroon (SIVcpz-Cam3, Cam4 and Cam5), 1 from a captive chimpanzee in the US (SIVcpz-US), 1 from a wild-caught animal of

Congolese (ex-Zaire) origin, intercepted by Belgian customs officers after illegal export from Kinshasa (SIVcpz-ant), and finally 1 from Tanzania (SIVcpz-Tan1) (23, 39, 50, 51, 72, 74, 81). With the exception of SIVcpz-Tan1, all the SIVcpz strains were identified in captive animals, captured at very young age. SIVcpz-Tan1 is the only sample from an adult wild-living chimpanzee and was obtained after the development of non-invasive methods to detect and characterize SIVcpz in fecal and urine samples (80, 81).

Chimpanzees are distributed across western and equatorial Africa and can be divided into four distinct subspecies according to mitochondrial DNA (mtDNA) sequences (38). The different subspecies are also geographically separated: *Pan troglodytes verus* is restricted to West Africa from southern Senegal to Ivory Coast, *Pan troglodytes troglodytes* is present across West central Africa from southern Cameroon to the Oubangui River in Congo, *Pan troglodytes schweinfurthii* lives in east central Africa including eastern Congo (DRC), Uganda, Ruanda, Burundi and Tanzania; and finally, *Pan troglodytes vellerosus* are genetically distinct chimpanzees restricted to a small geographic region between the Cross-River in Nigeria and the Sanaga River in Cameroon (43).

Natural infection with SIVcpz, is only identified in *Pan troglodytes troglodytes* and *Pan troglodytes schweinfurthii*. One SIVcpz (SIVcpz-cam4) infection was reported in *Pan troglodytes vellerosus* but the sequence was very similar to SIVcpz-Cam3 from his naturally infected *P.t.troglodytes* cagemate, suggesting that the vellerosus chimpanzee became infected in captivity (23). *P. t. verus* were largely exported from West Africa to primate centers in Europe and the US, and several hundred animals have been tested for HIV cross-reactive antibodies. All samples were negative, suggesting that *Pan troglodytes verus* is probably not infected with an HIV-1 related virus in the wild (78). Interestingly, the SIVcpz strains from West Central African and East Central African chimpanzees form two distinct clusters in the HIV-1/SIVcpz lineage with SIVcpz-ant and SIVcpz-Tan1 from *P.t. schweinfurthii* being most divergent from the HIV-1 group M, N and O strains (80, 81) (Fig. 2a and 2b). Since all three groups of HIV-1 are significantly more closely related to the SIVcpz strains from West Central chimpanzees, the cross-species transmissions giving rise to HIV-1 most probably all occurred in West central Africa (85). The greatest diversity of group M strains is in West equatorial Africa, (76, 102) close to the area inhabited by West Central chimpanzees, consistent with this being the region of group M origin. HIV-1 groups N and O viruses are also restricted to West central Africa (4, 73). Moreover, chimpanzee and group N human viruses from Cameroon form a unique subcluster in phylogenetic trees of *env* and *nef* regions (39, 89). HIV-1 groups M, N and O thus probably represent 3 distinct cross-species transmissions of SIVcpz (85).

The prevalence of SIVcpz infection in the wild is not yet very well known. The majority of the animals tested were captured as infants, and these infections are most probably the result of vertical transmission and do not reflect prevalences among adult animals. Chimpanzees are a highly endangered species and sampling in the wild is only possible through non-invasive methods, as those developed to detect the SIVcpz-Tan 1 strain (80, 81). The only study among wild chimpanzees, from east (*P.t. schweinfurthii*) and west (*P.t. verus*) African chimpanzees, reported low prevalences (81). However, additional field studies are necessary on a larger number of animals, and should also include *P.t.troglodytes* and *P.t.vellerosus* for which no data are available.

The absence of SIVcpz infection in *P.t. verus* suggests that chimpanzees became infected with a lentivirus after the geographic isolation of the west African chimpanzees. Low prevalences can also be the result of the declining chimpanzee population, and habitat fragmentation, which could even lead to extinction of SIVcpz in certain communities (81).

The SIVagm lineage

African green monkeys are widely dispersed over sub-Saharan Africa and have been classified as a separate genus (*Chlorocebus*) which is comprised of four species in different geographic regions: grivets (*Chlorocebus aethiops*) live in Ethiopia and Sudan, vervets (*Chlorocebus pygerythrus*) can be found from East to South Africa, tantalus monkeys (*Chlorocebus tantalus*) are prevalent in Central Africa, and sabaeus monkeys (*Chlorocebus sabaeus*) are restricted to West Africa (43). The first SIVagm viruses were isolated from African green monkeys from Kenya, but subsequently SIVs have been char-

acterized also in the other species originating from other regions in Africa (1, 5, 6, 30, 35, 37, 47, 52, 54, 60, 64, 90). Sequence and phylogenetic analysis showed that each of the four species carry their own species-specific SIV, because the viruses from each of the four African green monkey species form four distinct monophyletic clusters, which are more closely related to each other than to other SIVs. These observations suggest that the distinct forms of SIVagm may have evolved in parallel to their hosts (1, 36, 64). According to their host species, the SIVagm isolates have been named SIVagmVer, SIVagmGri, SIVagmSab, and SIVagmTan for vervets, grivets, sabaeus and tantalus monkeys respectively.

SIVagmSab from sabaeus monkeys has a mosaic genome structure. Parts of the genome (3' end of *gag* and 5' end of *pol*) cluster with the SIVsm/HIV-2 lineage whereas the rest of the genome groups with the SIVagm lineage (52). This indicates, as already mentioned above, that recombination between divergent SIVs occurred during the evolution of SIVagmSab.

High seroprevalences and a high genetic diversity have been observed in the wild among the different African green monkey populations (14, 64, 67) but also in captivity. SIVagm infection has little or no impact on the survival of the monkeys, and the virus is mainly transmitted by sexual contact and rarely by traumas or from mother to offspring (55).

The SIVsyk lineage

SIVsyk has been identified and isolated from Sykes monkeys (*Cercopithecus albogularis*) from Kenya. So far, only one full-length sequence of SIVsyk has been described and characterized (46). Similar to African green monkeys and sooty mangabeys, Sykes monkeys exhibit also a high SIV seroprevalence in the wild (33).

The SIVlhoest lineage

This lineage includes viruses isolated from three different primate species, l'Hoest monkeys (*Cercopithecus lhoesti*), sun-tailed monkeys (*Cercopithecus solatus*), and mandrills (*Mandrillus sphinx*). SIVmndGB1, from a mandrill in Gabon, was for more than ten years the only representative from this lineage. SIVmnd was described in 1988, whereas the viruses from sun-tailed and l'Hoest monkeys were reported in 1999 (10, 45, 95, 96). Mandrills represent a genus from the *Papionini* tribe, whereas l'Hoest and sun-tailed monkeys are representatives from the *l'hoesti* superspecies from the *Cercopithecini* tribe (43). The *C.l'hoesti* superspecies comprises 3 primate species: (1) l'Hoest monkeys (*Cercopithecus l'hoesti*) living from eastern Congo (DRC) to western Uganda, Ruanda and Burundi, (2) sun-tailed monkeys (*Cercopithecus solatus*), restricted to the evergreen forest area in central Gabon and (3) Preuss's monkeys (*Cercopithecus preussi*) in southeast Nigeria to southwest Cameroon (43). The close relationship of SIVlhoest and SIVsun parallels the close relationship between their two host species, and are an additional example of host-dependent evolution (9, 10). It will be interesting to determine whether Preuss's monkeys, are also naturally infected with SIV, and how their virus might be related to SIVlhoest and SIVsun. L'Hoest monkeys appear to be infected with SIV at quite high frequencies in the wild (9).

Two different types of SIVmnd: SIVmnd-1 and SIVmnd-2

The first SIVmnd (SIVmndGB1) was identified in 1988 in a wild-born mandrill residing in a primatology center in Gabon (CIRMF) (96). Recently, a second highly divergent SIVmnd virus was described from an other wild-born SIV positive mandrill residing in the same primate center in Gabon (91). The genomic organization differs significantly between the two viruses which are now designated as SIVmnd-1 and SIVmnd-2, for the SIVmndGB1 and the new SIVmndGB14 prototype strains, respectively. SIVmnd-2 has a *vpx* gene, as observed in the SIVsm/HIV-2 lineage. SIVmnd-1 and SIVmnd-2 are only related to each other in the *env* and *nef* region (Fig 2b). In the *gag-pol* region SIVmnd-2 is most closely related to SIVrcm from red-capped mangabeys (see below for more details on SIVrcm) and both, SIVmnd-2 and SIVrcm cluster with the HIV-1/SIVcpz lineage in *pol* (Fig 2a). On the other hand, SIVmnd-1 is close to the SIVl'hoest/SIVsun lineage in *gag-pol*. The distribution of the two types of SIVmnd is geographically separated by the Ogooué river in Gabon, SIVmnd-1 viruses were exclusively identified in mandrills from central and southern Gabon, and SIVmnd-2 in monkeys from northern and western Gabon and in Cameroon (91, 93).

The distribution of mandrills is restricted to West central Africa, and ranges from the south of the Sanaga River in Cameroon, to the north of Congo (43). The ranges of mandrills and sun-tailed monkeys thus overlap in Gabon. The presence of closely related viruses in such distantly related hosts suggests that cross-species transmission must have occurred at some point in the past between sun-tailed monkeys, or between the ancestor of sun-tailed and l'Hoest monkeys, and mandrills.

Characterization of more SIVs from mandrills in different geographic locations will be necessary to find out the exact origin of SIVs in mandrills. Importantly, the observation in wild mandrills illustrate that primate species in the wild can harbor two types of SIVs which both spread successfully. Prevalences of both types of viruses are apparently high among wild mandrill populations (91).

Figure 3a illustrates the distribution of the l'Hoesti superspecies and mandrills, and the distribution of the SIVmnd-1 and mnd-2.

The SIVcol lineage

SIVcol from mantled guerezas in Cameroon was the first primate lentivirus identified in the *Colobinae* subfamily. During a sero-survey in Cameroon, 25 wild-born Colobus monkeys (*Colobus guereza*) were screened and 7 were identified with HIV/SIV cross-reactive antibodies. Only one full-length genome sequence is available, SIVcolCGU1 (26). Genetic and phylogenetic analyses confirmed that SIVcol is genetically distinct from all other previously characterized SIV/HIV isolates and clusters independently, forming a separate lineage in the current classification. The fact that SIVcol is very divergent from all known SIVs may reflect divergence of the host lineage. Colobids split off from other Old World monkeys at least 11 million years ago (70).

SIVrcm from red-capped mangabeys

SIVrcm has been isolated from red-capped mangabeys living in Nigeria and Gabon (11, 41). For only one SIVrcm strain, NG411, the full length genome was sequenced (11). But partial sequencing suggests that red-capped mangabeys from distant geographic locations harbor a common SIV lineage and confirms thus that these animals are the natural host for SIVrcm. SIVrcm shares the genomic organization characteristic of the SIVsm/HIV-2 lineage, i.e. presence of a *vpx* gene. However, phylogenetic

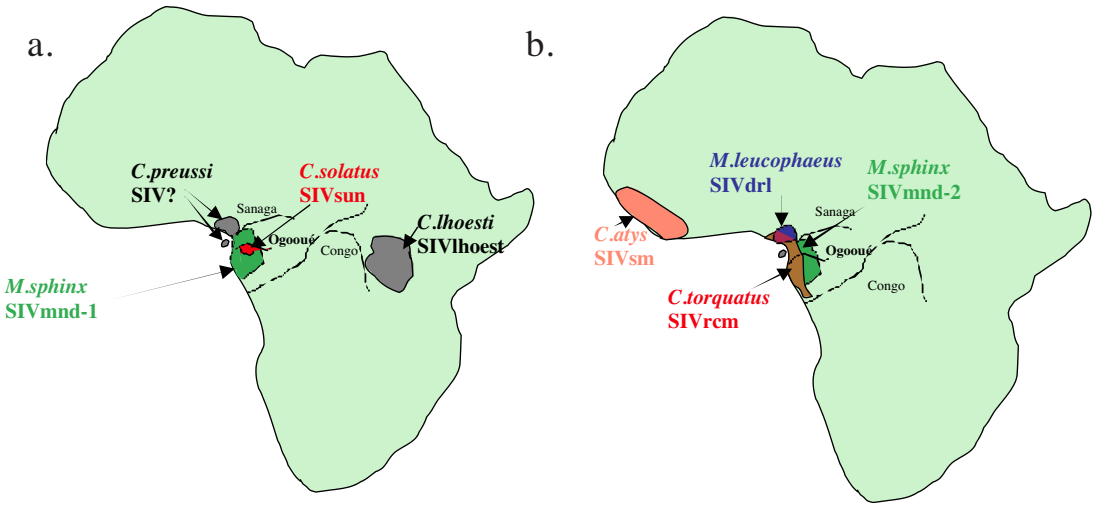

Figure 3: Geographic distribution of African primates infected with closely related viruses for the entire or partial SIV genome.
(a). The distribution of the 3 different primates belonging to the l'hoesti superspecies and mandrills infected with the SIVmnd-1 strains. SIVmnd-1 is closely related to SIVsun. The geographic distribution of SIVmnd-1 and SIVmnd-2 in mandrills is separated by the Ogooué River in Gabon.
(b) The distribution of drills and mandrills infected with the SIVmnd-2 strains and mangabeys for which SIV infection was documented. SIVrcm is closely related to SIVdrl and SIVmnd-2 in pol.

analysis, showed that SIVrcm was quite divergent from SIVsm. In *pol*, SIVrcm was most closely related to SIVcpz and SIVmnd-2 from mandrills (Figure 2a), whereas in other parts of the genome the virus clusters with SIVagm-sab or SIVsm, suggesting a history of recombination between different SIV lineages. Although the virus is only distantly related to SIVsm, it still clusters with SIVsm and SIVagm in the *env* and *nef* region (Fig 2b).

Red-capped and sooty mangabeys are phylogenetically closely related species, both belonging to the *Cercocebus* genus. Mangabeys (*Cercocebus sp*) are related to mandrills, and most probably derive from a common ancestor (43, 57). Only the *vpx* gene is common to the SIVs isolated from 2 representatives of the *Cercocebus* genus and for SIVmnd-2 from mandrills. This could suggest also a common SIV ancestor for this group of primates, however, the divergence between SIVsm and SIVrcm suggests a different evolution among different mangabeys. SIVrcm and SIVmnd-2 share more common genomic regions than SIVrcm and SIVsm. The geographic distribution of red-capped mangabeys does overlap that of mandrills and drills allowing thus possible cross-species transmissions between them, whereas sooty mangabeys are restricted since a long period to West Africa only (Figure 3b).

SIVgsn from greater spot-nosed monkeys

During a large seroprevalence survey of wild-born monkeys in Cameroon, 27 out of 165 greater spot-nosed monkeys (*Cercopithecus nictitans*) had antibodies cross-reacting with HIV antigens (71). For 2 animals, 99CM71 and 99CM166, the complete SIVgsn genomes were successfully amplified and sequenced (27). Together with SIVsyk, SIVgsn represents the second virus isolated from a monkey belonging to the *C.mitis* group of the *Cercopithecus* genus (43). Full-length genome sequence analysis of two SIVgsn strains, SIVgsn-99CM71 and SIVgsn-99CM166, revealed that despite the close phylogenetic relationship of their hosts, SIVgsn was highly divergent from SIVsyk. Surprisingly, the genomic organization of SIVgsn was similar to that of SIVcpz and HIV-1, i.e it harbored the additional accessory gene *vpu* specific to the members of this lineage (Figure 1). The *vpu* genes from SIVgsn-99CM71 and SIVgsn-99CM166 were closely related to each other with 72% identity but show less than 35% identity only with *vpu* from HIV-1 or SIVcpz. This is not surprising because even between Vpu from SIVcpz or HIV-1 group M or O, a high variability has been documented. Nevertheless, the position in the genome of this ORF fragment, as well as the hydropathy profile displayed by its deduced protein which is similar to Vpu, allowed to identify this ORF as a *vpu* gene(27). SIVgsn is therefore the first SIV isolated from a lower monkey species to have a *vpu* gene.

Phylogenetic analyses against the previously described HIV/SIV lineages on various regions of the viral genome indicated that SIVgsn might be a mosaic of sequences with different evolutionary histories. SIVgsn was related to SIVsyk in Gag and part of Pol and related to SIVcpz in Env (Figure 2a and 2b). When comparing the two SIVgsn Env sequences with SIVcpz, a remarkable conservation was seen in the V3 loop (27).

The presence of a *vpu* gene in SIVgsn and its relatedness to SIVcpz in the envelope suggest a link between SIVgsn and SIVcpz. Habitats of both subspecies of chimpanzees infected by SIVcpz have overlapping geographic ranges with greater spot-nosed monkeys and other monkey species thus allowing cross-species transmission and recombination between coinfecting viruses .

PARTIALLY CHARACTERIZED PRIMATE LENTIVIRUSES

Figure 4 shows a phylogenetic tree, based on partial pol sequences, illustrating the diversity among primate lentiviruses and the phylogenetic relationship between the partially characterized SIVs to each other and to the above described SIV lineages in this small part of the genome. The tree was derived from a 650 bp fragment in the integrase region of the pol gene. In this small region, the clusters observed are not always significantly supported by high bootstrap values.

SIV from patas monkeys and baboons

Based on partial sequences, SIV infection has been described in Patas monkeys (*Erythrocebus patas*) in West Africa, chacma baboons (*Papio ursinus*) in South Africa and a yellow baboon (*Papio hamadryas cynocephalus*) in Tanzania (15, 53, 100). Phylogenetic analysis revealed that the SIVs in

these monkeys were closely related to SIVs derived from the local sympatric species of African green monkeys. These findings together with the low prevalences in wild animals suggested a simian to simian cross-species transmission in these wild ranging animals (58).

SIVdrl

Recently SIV was obtained from drills (*Mandrillus leucophaeus*) from Nigeria and Cameroon (22, 91). Drills are closely related to mandrills but their actual distribution is limited to West central Africa, and does not overlap at all. Based on partial pol and *env* sequences, SIVdrl was found to be closely related to SIVmnd-2 and not to SIVmnd-1 (91). Similarly as SIVmnd-2, SIVdrl was most closely related to SIVrcm and SIVcpz in pol, and to SIVmnd-1 in *env*. But only full-length sequence will allow to conclude whether host dependant evolution occurred in the *Mandrillus* genus for the SIVmnd-2 and SIVdrl viruses.

SIVtal

Partial *pol* sequences are described for SIVtal from the 2 different talapoin species, *Miopithecus ogouensis* from Cameroon and *Miopithecus talapoin* from a zoo animal (69, 71). Both SIVs formed together a species specific monophyletic cluster roughly equidistant from all other SIVs in this region of the *pol* gene. The recently described SIVtal sequences from animals from Cameroon confirm the existence of this lineage in the wild (71).

SIV from different Cercopithecus species

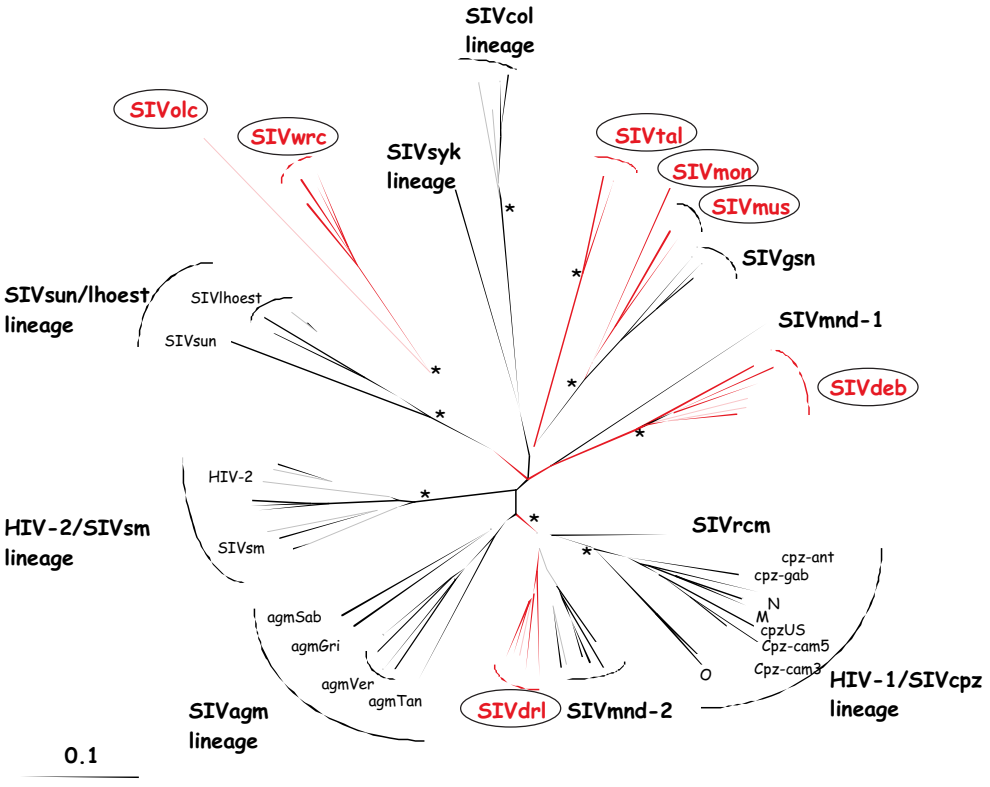

Figure 4: Evolutionary relationship of partially characterized SIVs in pol (in elipses) to the previously described SIV lineages. The unrooted tree was derived by neighbor-joining analysis of partial pol nucleotide sequences. Branch lengths are drawn to scale (the bar indicates 10% divergence). The asterisk at the nodes indicate bootstrap values supporting the cluster to the right with values >80%.

Partial *pol* sequences have also been reported for several guenons, more precisely from de Brazza monkeys (*Cercopithecus neglectus*) (SIVdeb), mona monkeys (*Cercopithecus mona*) (SIVmon) and mustached monkeys (*Cercopithecus cephus*) (SIVmus) from Cameroon as well as from Blue monkeys (*Cercopithecus mitis*) (SIVblu) from Kenya and Wolf's (*Cercopithecus wolfi*) (SIVwol) and red tailed (*C.ascanius*) monkeys from the Democratic Republic of Congo (DRC) (13, 71, 82). Based on the partial sequences from these viruses, two clusters can be identified among the corresponding SIVs: SIVdeb, SIVblu and SIVwol seem to cluster with the SIVsyk lineage, and SIVmon and SIVmus cluster with SIVgsn (13, 32, 71). Near full-length genome sequencing of SIVmon and SIVmus confirmed this close relationship to SIVgsn in other parts of the genome, as well as the presence of a *vpu* gene (24). As mentioned above, a *vpu* gene is also reported for SIVwol but the envelope seems not to cluster with the HIV-1/SIVcpz lineage as is the case for SIVgsn, SIVmon and SIVmus (32). Only full-length genome sequences will make it possible to determine their exact phylogenetic position in the primate lentivirus family.

SIVagi

Recently, SIVagi has been isolated from an agile monkey (*Cercocebus agilis*) in Cameroon . The partial characterization shows that SIVagi is closely related to SIVrcm in *gag* and *pol*. The prevalences in the wild can reach 20% (65).

SIV from West African Colobids.

The living African colobids are represented by 3 genera, *Colobus* or black and white colobus, *Piliocolobus* or red colobus and *Procolobus* or olive colobus (43). All contemporary species of the African Colobids are restricted to the tropical and mountain forest belt of Africa (57).

West African Colobids from the Taï National Park, located in the south-west of Ivory Coast near the border with Liberia, for which blood was obtained between 1997 and 2000 were tested for the presence of SIV infection. This park is the largest remaining area of primary forest in West Africa. Blood was obtained from 13 west African Colobids, and HIV cross-reactive antibodies were observed in 5/10 western red colobus monkeys (*Piliocolobus badius*), 1/2 olive colobus (*Procolobus verus*) and 0/1 black and white colobus (*Colobus polykomos*). Phylogenetic analysis of a 650 bp fragment in *pol* confirmed SIV infection (Figure 4) and showed that viral sequences from western red (SIVwrc) and olive (SIVolc) colobus each formed species specific monophyletic clusters. The new SIVs obtained from 2 different genera in the *Colobinae* subfamily were more closely related to each other than to the other SIVs and were not at all related to the SIVcol strain obtained from a mantled guereza (*C.guereza*) from Cameroon. A 2000 bp fragment in *pol* was then obtained for a representative SIV from each species and SIVwrc and SIVolc showed 59.8% amino acid identities in this part from the genome, the lowest homology was seen with the SIVcol strain, with 50.8% amino acid identities only (25).

The 3 African genera from the *Colobinae* subfamily thus are naturally infected with SIV at relatively high prevalences. At least one representative from each genus is infected, *Colobus guereza* in Cameroon, *Piliocolobus badius* and *Procolobus verus* in Ivory Coast. It seems that these viruses have not evolved in a host-dependent fashion at the level of the *Colobinae* subfamily, since representatives of the 3 genera do not cluster together in the region studied. However, full-length genome sequencing of the new SIVolc and SIVwrc sequences is necessary to see to what extent they are pure or recombinant SIVs. We have also to take into account the geographical origin from the SIV harboring species which we are comparing. It is important to note that the African colobid species are reflected by their geographic distribution, e.g. the olive colobus is a relict species confined to the forest of West Africa and red colobus (*Piliocolobus sp*) once ranged all over the forested areas from Africa, but their regional differentiation shows that their scattered distribution has existed since a long time (43, 57).

In order to understand the evolution of SIVs in the *Colobinae* subfamily, it is important to identify and compare SIVs from *Colobus* and *Piliocolobus* species from West, Central and East Africa, to find out whether co-evolution between viruses and host or cross-species transmissions among different colobids occurred. Colobus monkeys share habitats with *Cercopithecus* species and with mangabeys, therefore an exchange of ancestral SIVs between these species could have been possible in the past.

Screening of the other sympatric species from the *Cercopithecinae* and *Colobinae* subfamilies is also required, ex. *Cercopithecus diana*, *Colobus polykomos* from the Taï forest to see whether they became infected with other co-habiting monkeys in the past.

CHARACTERIZATION OF NOVEL SIVS PROVIDES NEW INSIGHTS IN THE OVERALL PRIMATE LENTIVIRAL EVOLUTION

Our understanding of the complex picture of the overall primate lentiviral evolution gains from characterization of novel SIVs. Retroviruses are known to be highly recombinogenic and several examples of mosaic genomes have been described within HIV(88). There is now evidence for at least five SIVs with discordant phylogenies when different genes are studied : SIVagm-Sab in sabaeus monkeys, SIVrcm in red-capped mangabeys, SIVmnd2 in mandrills, SIVdrl in drills and SIVgsn in greater spot-nosed monkeys (11, 27, 52, 91). This indicates that cross-species transmissions and recombinations have existed since the beginning of the evolution of primate lentiviruses. Important to note is that identification of "pure lineages" or "recombinants" is mainly a function of chronological findings. The first characterized viruses are usually identified as pure. Thus, if one virus previously determined as "pure" has been derived in reality from early recombinations between ancestral viruses, "parental" viruses characterized afterwards will be considered to be recombinants. Therefore recently characterized viruses like SIVrcm, SIVmnd-2 and SIVgsn are actually considered as complex recombinants, likely resulting from ancient recombination events involving ancestors of the current SIVcpz viruses. But based on the behaviour of these primates, it seems more plausible that chimpanzees acquired SIV infection from other monkeys rather than the opposite because they are known to hunt and eat several small monkeys (16, 92, 97). Chimpanzees have overlapping geographic ranges with the above mentioned monkeys, allowing thus cross-species transmission, superinfection and potential recombination between coinfecting viruses.

Therefore, based on primate behaviour and HIV/SIV phylogeny an alternative hypothesis may be proposed for the genome structure of SIVcpz and SIVgsn. In the envelope SIVgsn clusters with the SIVcpz/HIV-1 lineage where it branches off before SIVcpzANT which itself is an outgroup of the SIVcpz/HIV-1 lineage and in *gag-pol* SIVgsn clusters with SIVsyk (Fig. 2a and 2b). When the envelope tree is considered without the SIVcpz/HIV-1 lineage, SIVgsn clusters with SIVsyk, similarly as in the *gag-pol* tree (Figure 2b). These observations are also confirmed by a diversity plot or bootscan analysis performed without the SIVcpz lineage. Therefore, it is thus possible that SIVgsn is a pure lineage related to SIVsyk, or recombinant between *vpu*-harboring SIVs related to SIVsyk. In this case, it remains possible that *vpu* has been acquired during the evolution of SIVgsn or from another SIV from monkeys sharing the same habitat, rather than resulting from recombination between an SIV from a Cercopithecus species and SIVcpz. Another possibility could be that SIVsyk lost the *vpu* gene during its evolution.

SIVcpz is a recombinant lineage

If we assume that SIVgsn is a pure lineage, that implies that SIVcpz occurred by recombination. Since the current prevalence of SIVcpz infection among chimpanzees seems low in captivity as well as in their natural habitat in Africa (23, 72, 74, 81) it has already been suggested that another, as yet unidentified primate species, could be the natural host for SIVcpz. Obviously, SIVgsn is not the immediate SIVcpz progenitor since the close relationship of SIVcpz and SIVgsn is restricted to Env. But given that chimpanzees, as mentioned above, are exposed to SIVs from other species by their predatory behaviour, it is possible that they have been infected and co-infected by different viruses and that this recombinant virus then successfully spread among chimpanzees and subsequently in humans.

Among all the species known so far for harboring an SIV, none carries an SIV that is closely related to SIVcpz across the entire genome. The recent identification of SIVrcm and SIVmnd2, which are closely related to SIVcpz in *pol* and the present identification of SIVgsn, which is closely related to SIVcpz in *env* support our hypothesis that the present SIVcpz may have been generated by recombination between ancestral SIVs from the *Cercopithecidae* family. Furthermore, bootscan and similarity

analysis of SIVcpz against representatives of the six major SIV lineages including SIVmnd2 or SIVrcm and SIVgsn confirm the recombinant structure of SIVcpz as we previously proposed (27, 6a). For example,the diversity analysis of SIVcpz-ant against the other SIV lineages in Figure 5 shows clearly that SIVcpz-ant is more closely related to SIVmnd-2 and SIVrcm in Gag-Pol and to SIVgsn in Env. SIVcpz most probably acquired a *vpu* gene from a lower monkey species. Figure 6 illustrates the habitats of chimpanzees and *Cercopithecus* species in which SIVs with a *vpu* gene were documented.

However chimpanzees are now a natural host of SIVcpz in the wild otherwise members of this same group of viruses would not be found throughout equatorial Africa (81) but the questions are: When, where and how chimpanzees became infected and with which and how many species? Additional characterizations of SIVs from other monkeys are needed to answer these questions.

CONCLUSIONS

The recent characterizations of SIVrcm, SIVgsn, and SIVmnd-2, ilustrate clearly that primate lentivirus evolution is very complex. Recombination between distant SIVs seems not exceptional, and this ultimately implies also that cross-species transmissions and superinfection with distant SIVs seem to occur frequently. In order to better understand which SIV lineage is pure or recombinant, a multidisciplinary approach is necessary between virologists, molecular and evolutionary biologists and primatologists. Knowledge of primate behaviour and past and recent geographic distribution of the different primate species could add important complementary information for the interpretation of SIV sequences.

Primatologists showed that monkeys living in tropical forests often form aggregations consisting of multiple species: 'poly-specific associations'. Data on primate behaviour can thus be useful to understand primate lentivirus evolution and to understand whether superinfections and subsequent viral recombinations are possible.

An important observation was the fact that mandrills can be naturally infected with two different SIVs, which both circulate among geographically separated groups of mandrills (91, 93). It will be important to find out whether this also exists among other primate species. Two virus types in one species suggest recent or past cross-species transmissions, and can thus be expected to occur among co-habiting primates for which contacts between the different species have been documented, either through predation, fighting for food or habitat or sexual contacts (e.g. wild guenons of different species hybridize easily (57)).

There is no doubt that primates are infected for a long period of time with SIVs and that there is co-evolution between viruses and hosts. This is clearly illustrated by the lack of AIDS-like disease and the continuous high-level replication of the virus in these African primates (31, 42, 49, 68, 79). But in order to find out to what extent host dependant evolution with a common SIV ancestor and/or cross-species transmissions between co-habiting primate species occurred, screening of geographic separated primate populations belonging to the same species will be necessary. For example, are greater spot-nosed monkeys, which are largely distributed across Africa, always infected with an SIV from the same lineage?

Screening from larger number of primates from different localities in Africa, will also allow to determine to what extent cross-species infection, superinfection and recombination occurred and to what extent this is still ongoing.

But most importantly, it is now well established that both groups of viruses giving rise to AIDS in humans appear to have resulted from several independent transmissions with SIVs from African primates (44, 85). We recently documented that humans are still exposed to a large variety of SIVs when they hunt and handle primates as bushmeat (71). Bushmeat hunting is not limited to chimpanzees and mangabeys, the majority of non-human primates in the bushmeat trade are represented by multiple *Cercopithecus* species, colobus monkeys, mandrills, drills etc. Bushmeat hunting, to provide animal protein for the family and as a source of income, is a longstanding common component of rural household economies in the Congo Basin, and more generally throughout sub-Saharan Africa (2). However, the bushmeat trade has increased in the last decades, due to the expanding logging industry in certain

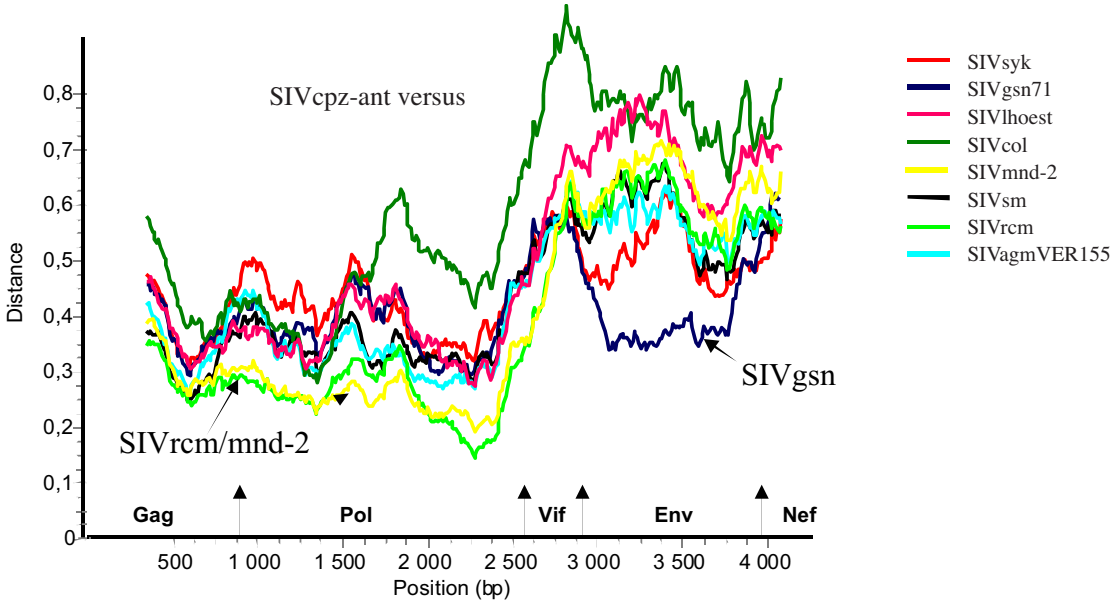

Figure 5: Diversity plot comparing SIVcpz-ant against representatives of the major lineages of the primate lentiviruses, i.e., SIVgsn, SIVmnd-2, SIVrcm, SIVsyk, SIVsm, SIVagm and SIVcol. Protein sequence difference is plotted for windows of 200 amino acids moved in steps of 10.

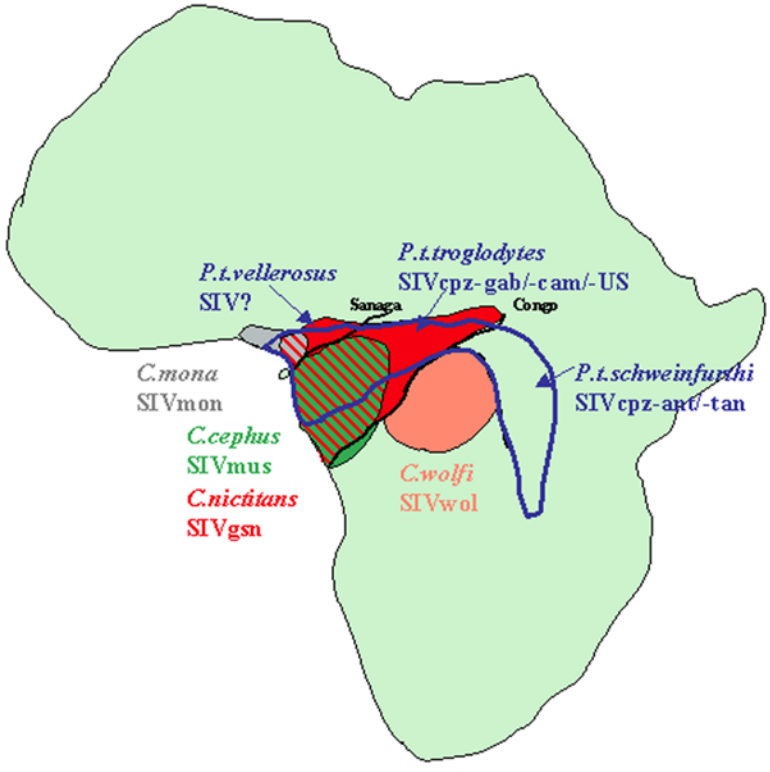

Figure 6 : Geographic distribution of African primates infected with an SIV harbouring a vpu gene: C.mona, C.nictitans, C.cephus, C.wolfi, P.t. troglodytes and P.t.schweinfurthii.

central African countries and demand of bushmeat in cities. Commercial logging has led to road constructions into remote forest areas, human migration, and the development of social and economic networks (including those of sex workers) which support this industry (3, 103) . Also, villages around logging concessions, have grown from a few hundred to several thousand inhabitants in just a few years. The number of people penetrating previously inaccessible forest areas increased significantly over the last decades. The socioeconomic changes which accompany the presence of logging industries in remote areas, combined with the high SIV prevalence and genetic complexity in wild living primates, would suggest that the magnitude of human exposure to SIV has increased, as have the social and environmental conditions that would be expected to support the emergence of new zoonotic infections.

One major public health implication is that, these SIVs are not recognized by commercial HIV-1/HIV-2 screening assays. As a consequence, human infection with such variants can initially go unrecognized and lead to another epidemic. The ability of several SIVs to infect human PBMCs *in vitro* suggests that these viruses could have the potential to infect human populations (10, 11, 45, 65, 72, 74, 75). Identification of SIVs in wild primates will help to elucidate the origins and evolution of HIV infection in man, but more importantly they can serve as sentinels by signaling which pathogens may be a risk for humans and allow the development of serological and molecular assays to detect zoonoses with other SIVs in humans.

As shown in this paper, the genetic diversity among non-human primate lentiviruses is extremely complex, already 33 species have been identified with serological evidence of SIV infection, and in 27, this was confirmed by partial or full-length genome sequencing. It is also clear now that cross-species transmission among non-human primates exists, which can evolve differently in their new host and which can lead also to new recombinants which can further spread. Recombination between newly introduced SIVs and circulating HIVs has thus also to be considered in humans and poses an additional risk for the outbreak of novel zoonoses.

More field studies are needed to understand the epidemiology, the diversity and evolution of primate lentiviruses. But due to destruction and degradation of the tropical forest areas by logging and agriculture pressure together with the increasing hunting pressure for the bushmeat trade, the majority of the primates are endangered species. Sampling of primates should therefore employ strategies that do not increase the further reduction of these animals. Recent studies showed that SIV sequences can be derived from fecal samples (61, 80, 81). Noninvasive sampling allows thus to conduct molecular epidemiological studies on primate lentiviruses in endangered species and are an oppurtunity for virologists, molecular and evolutionary biologists and primatologists to combine their expertise in order to better understand primate lentivirus evolution.

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