**env** Sequences of Simian Immunodeficiency Viruses from Chimpanzees in Cameroon Are Strongly Related to Those of Human Immunodeficiency Virus Group N from the Same Geographic Area

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Human immunodeficiency virus type 1 (HIV-1) group N from Cameroon is phylogenetically close, in env, to the simian immunodeficiency virus (SIV) cpz-gab from Gabon and SIVcpz-US of unknown geographic origin. We screened 29 wild-born Cameroonian chimpanzees and found that three (Cam3, Cam4, and Cam5) were positive for HIV-1 by Western blotting. Mitochondrial DNA sequence analysis demonstrated that Cam3 and Cam5 belonged to Pan troglodytes troglodytes and that Cam4 belonged to P. t. vellerosus. Genetic analyses of the viruses together with serological data demonstrated that at least one of the two P. t. troglodytes chimpanzees (Cam5) was infected in the wild, and revealed a horizontal transmission between Cam3 and Cam4. These data confirm that P. t. troglodytes is a natural host for HIV-1-related viruses. Furthermore, they show that SIVcpz can be transmitted in captivity, from one chimpanzee subspecies to another. All three SIVcpz-cam viruses clustered with HIV-1 N in env. The full Cam3 SIVcpz genome sequence showed a very close phylogenetic relationship with SIVcpz-US, a virus identified in a chimpanzee captured nearly 40 years earlier. Like SIVcpz-US, SIVcpz-cam3 was closely related to HIV-1 N in env, but not in pol, supporting the hypothesis that HIV-1 N results from a recombinant event. SIVcpz from chimpanzees born in the wild in Cameroon are thus strongly related in env to HIV-1 N from Cameroon, demonstrating the geographic coincidence of these human and simian viruses and providing a further strong argument in favor of the origin of HIV-1 being in chimpanzees.

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Human immunodeficiency virus type 1 (HIV-1) groups M, N, and O are considered to result from at least three independent introductions of simian immunodeficiency virus (SIV) cpz from chimpanzees into the human population (23). Four subspecies of chimpanzees are currently recognized: Pan troglodytes verus and simian viruses and providing a further strong argument in favor of the origin of HIV-1 being in chimpanzees.
TABLE 1. Reactivity of the sera from three SIVcpz-infected chimpanzees of Cameroon with distinct V3 and gp41 reference peptides

<table>
<thead>
<tr>
<th>Peptide and virus group</th>
<th>Serum reactivity</th>
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<tbody>
<tr>
<td></td>
<td>Group M</td>
</tr>
<tr>
<td>V3</td>
<td></td>
</tr>
<tr>
<td>Group M</td>
<td>1.54</td>
</tr>
<tr>
<td>Group O</td>
<td>0.11</td>
</tr>
<tr>
<td>Group N</td>
<td>0.11</td>
</tr>
<tr>
<td>SIVcpz-gab</td>
<td>0.11</td>
</tr>
<tr>
<td>gp41</td>
<td></td>
</tr>
<tr>
<td>Group M</td>
<td>1.60</td>
</tr>
<tr>
<td>Group O</td>
<td>1.25</td>
</tr>
<tr>
<td>Group N</td>
<td>0.22</td>
</tr>
<tr>
<td>SIVcpz-gab</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* The assays were based on the EIA technique as previously described (16).
  * The peptide is based on an HIV-1 subtype A sequence.
  * Bold type indicates strong reactivity with the peptide.

The sera of Cam3, Cam4, and Cam5 were further tested with a peptide-based enzyme immunoassay (EIA) (15). All three showed strong cross-reactivity with SIVcpz-gab and HIV-1 N peptides (Table 1), a pattern resembling that observed for sera from HIV-1 N-infected patients (23). The Cam5 serum was also strongly reactive with an HIV-1 group M Env peptide, possibly on account of the young age at sampling (26).

Virus isolation was attempted by means of coculture with phytohemagglutinin-stimulated human peripheral blood mononuclear cells (PBMC) from HIV-negative blood donors (23). Virus production was detected after 9 days of culture in each case, confirming that all three chimpanzees were infected.

For Cam5 it was clear that the infection occurred in the wild since the sampling was performed immediately after its arrival at the zoo, and the animals could be associated with an acquired immunodeficiency syndrome.

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For Cam5 it was clear that the infection occurred in the wild since the sampling was performed immediately after its arrival at the zoo, and the animals could be associated with an acquired immunodeficiency syndrome.
None of the three Cameroonian sequences was clearly related to HIV-1 M or HIV-1 O. Interestingly, SIVcpz-cam3 and SIVcpz-cam5 were only slightly more closely related to each other than to SIVcpz-US, even though the latter had infected its host nearly 40 years earlier (4).

We then analyzed the full-length genome (9,155 bp) of one Cameroonian SIVcpz virus (SIVcpz-cam3) by using the sequence approach described above. The SIVcpz-cam3 genome displayed the genomic organization typical of SIVcpz and HIV-1. Analysis of deduced amino acid sequences indicated that all the genes studied potentially encoded functional proteins. The highest nucleotide identity was always observed with SIVcpz-US (83% in pol and 76% in env). Phylogenetic analysis based on the entire gag, pol, and env genes confirmed the clustering of SIVcpz-cam3 and SIVcpz-US throughout their genomes (Fig. 1a and data not shown). These data demonstrate that viruses closely related to SIVcpz-US are currently circulating in wild-living chimpanzees in Cameroon.

The nucleotide identities of SIVcpz-cam3 with HIV-1 N and SIVcpz-gab were also high (respectively, 79 and 80% in pol and 73 and 69% in env). We therefore scanned the HIV-1 N genome with the recombinant identification program (Fig. 2 and data not shown). Significant similarity, as indicated by sites with statistical confidence above 90%, was observed between HIV-1 N YBF30 and SIVcpz-cam3 in two short stretches in gag (99 sites in total), a 300-bp region in env, and a long fragment in env (1,210 bp). A long stretch of significant homology was therefore found only in env. The regions of higher identity are visualized in the similarity plot (Fig. 2). Phylogenetic analysis based on the entire env gene confirmed the clear clustering of SIVcpz-cam3 with HIV-1 N YBF30, with a highly significant bootstrap value (100%) (Fig. 1b). SIVcpz-cam3 and HIV-1 N YBF30 branched together...
with the same high bootstrap value (100%) in the trees based on the amino acid sequences of env (data not shown). The bootstrap value for gag was lower (82% with the codon-based nucleotide alignment and <50% with the amino acid alignment [data not shown]). No such clustering was observed for pol (Fig. 1c); indeed, HIV-1 N YBF30 rather formed an independent branch closely related to HIV-1 M, as previously reported (23). The clustering of HIV-1 N with group M viruses in pol is supported by highly significant bootstrap values in the trees based on both nucleotide alignment (99%, Fig. 1c) and amino acid sequence alignment (98% [data not shown]). The different branching orders of HIV-1 N observed according to the gene analyzed could be explained by ancestral recombination events as previously suggested (4, 12).

Since two distinct chimpanzee subspecies are present in Cameroon (6, 17), we determined the subspecies of Cam3, Cam4, and Cam5 by mitochondrial DNA (mtDNA) sequence analysis. DNA of the mitochondrial control region (D loop) was amplified from uncultured PBMC of Cam3 and Cam4. Previously described primers (1) were used for PCR amplification and sequence determination of the PCR product. For Cam5, only genomic DNA from a 9-day coculture of chimpanzee PBMC with human PBMC was available. We therefore designed primers that specifically amplify either human or chimpanzee mtDNA. For amplification of chimpanzee mtDNA we designed the primers mtcpzs (5′-CTATAAGTGATTTGCTTATTGAATT-3′) and mtcpzas (3′-CTATCTGAGGGGGCACTCAG-5′). Human mtDNA was amplified as a control with the primers mthums (5′-CTGACTACGCTACCTAGCTG-3′) and mthumas (5′-CTATCTGAGGGGGCTCATCCA-3′).

Phylogenetic analysis of mtDNA from the three source chimpanzees in our study showed that two animals (Cam3 and Cam5) belonged to P. t. troglodytes (Fig. 3). The third seropositive chimpanzee (Cam4) did not cluster with P. t. troglodytes but with the P. t. vellerosus lineage (Fig. 3). This classification of Cam4 based on the mtDNA sequence fits with the geographic origin of the chimpanzee (Southwest Cameroon). This is the first time that a West African chimpanzee has been shown to be infected by SIVcpz. Serological and genetic analysis suggest that Cam4 was infected by Cam3 in captivity, although we cannot totally exclude that the virus crossed from Cam4 to Cam3. It is the first documented case of cross-subspecies transmission of SIVcpz in captivity. As both apes were males, transmission might have occurred through biting, a com-
mon route of lentivirus transmission in animals (18), and not by sexual contact. These data also confirm that *P. t. troglodytes* is a natural host of HIV-1-related viruses, since Cam5, and maybe also Cam3, was infected in the wild (4). Both animals were captured at a very young age, as were all four other SIVcpz-infected chimpanzees reported in the literature (4, 19, 20). As two of the 29 infants tested here were apparently infected in the wild, the seroprevalence of SIVcpz in free-living adult chimpanzees may indeed be higher than previously assumed (4).

An important implication of this study is that the area of HIV-1 N endemicity coincides with the natural habitat of chimpanzees carrying HIV-1 N-related SIVcpz, providing the most persuasive evidence that SIVcpz is the evolutionary ancestor of HIV-1.

The similarity between SIVcpz from *P. t. troglodytes* and HIV-1 N was, however, only seen in *env*. HIV-1 N therefore most likely resulted from an ancestral recombination event between viruses related to SIVcpz-cam/US and HIV-1 M (4). The recombination probably occurred in a chimpanzee (4), although one cannot entirely exclude the possibility that it occurred in a human. Only the discovery of such a recombinant virus in chimpanzees or of an HIV-1 N virus related to SIVcpz-cam/US throughout its genome can settle the matter. Additional HIV-1 N and SIVcpz sequences are needed to determine in which species and where exactly in the genome recombination took place.

HIV-1 groups M, N, and O are all circulating in Cameroon (16). It is therefore surprising that all Cameroonian SIVcpz sequences so far identified show a particular relationship with HIV-1 N, but not with HIV-1 M, as the latter has a considerably higher prevalence than N and O in Cameroon (16). It is also surprising that none is related to HIV-1 group O, as Cameroon is currently the epidemiological center of this group (16). The possibility that HIV-1 derives from species other than chimpanzees is unlikely, but it cannot be totally ruled out. Another explanation might be that group M- and group O-related viruses no longer exist in chimpanzees. Studies of wild-born chimpanzees are too limited to exclude, however, the presence of HIV-1 M- or O-related viruses in animals of other geographic regions or in subspecies distinct from *P. t. troglodytes*. The existence of divergent viruses (SIVcpz-gab and SIVcpz-cam/US) within chimpanzees of the same subspecies (*P. t. troglodytes*) but from distinct geographic origins, as well as the presence of the highly divergent SIVcpz-ant in another subspecies of East Africa (*P. t. schweinfurthii*), indicates indeed that chimpanzees in Eastern and Central Africa may harbor a broad spectrum of SIVcpz-type viruses. West African chimpanzees might harbor SIVcpz as well. Indeed, we report here
on a case of cross-subspecies transmission between a Central and a West African chimpanzee in captivity. As both subspecies occur in Cameroon, it is easily conceivable that such transmissions occurred in the past between wild animals.

Although HIV-1 group M- and O-related viruses have not been identified yet in chimpanzees (or in any other nonhuman primate species), these African apes are clearly the best candidates for explaining the origin of HIV-1. HIV-2, as is generally agreed, originates from SIVsm present in a West African nonhuman primate, the sooty mangabey (5). The ancestors of SIVcpz and SIVsm, in contrast, are still totally unknown. The reservoir of SIVcpz and SIVsm (if it still exists) would be a further key to understanding the origin of these primate lentiviruses.

In conclusion, we demonstrate that SIVcpz and closely related HIV-1 viruses currently cocirculate in both wild chimpanzees and humans from the same geographic area, providing a further step towards the missing link between HIV-1 and related viruses in nonhuman primates.

**Nucleotide sequence accession numbers.** SIVcpz-cam sequences have been deposited at the GenBank database under accession no. AF115393 to AF115395 and AF135498. Mitochondrial sequences of C2, C4, and C5 have been deposited at the GenBank database under accession no. AF13495 to AF134947.

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**REFERENCES**


