Chimpanzee Reservoirs of Pandemic and Nonpandemic HIV-1

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Human immunodeficiency virus type 1 (HIV-1), the cause of human acquired immunodeficiency syndrome (AIDS), is a zoonotic infection of staggering proportions and social impact. Yet uncertainty persists regarding its natural reservoir. The virus most closely related to HIV-1 is a simian immunodeficiency virus (SIV) thus far identified only in captive members of the chimpanzee subspecies Pan troglodytes troglodytes. Here we report the detection of SIVcpz antibodies and nucleic acids in fecal samples from wild-living P. t. troglodytes apes in southern Cameroon, where prevalence rates in some communities reached 29 to 35%. By sequence analysis of endemic SIVcpz strains, we could trace the origins of pandemic (group M) and nonpandemic (group N) HIV-1 to distinct, geographically isolated chimpanzee communities. These findings establish P. t. troglodytes as a natural reservoir of HIV-1.

Since the first detection of an HIV-1–related lentivirus in chimpanzees (1, 2), this species has been suspected as the source of the human AIDS pandemic. However, a crucial missing link in the chain of evidence implicating SIVcpz in the origin of HIV-1 and AIDS has been the absence of a recognizable virus reservoir in wild-living apes. Chimpanzees (Pan troglodytes) are classified into four subspecies on the basis of differences in mitochondrial DNA sequence (3); P. t. verus in west Africa; P. t. vellerosus in Nigeria and northern Cameroon; P. t. troglodytes in southern Cameroon, Gabon, and the Republic of Congo; and P. t. schweinfurthii in the Democratic Republic of Congo and countries to the east (Fig. 1). Two of these subspecies, P. t. troglodytes and P. t. schweinfurthii, are known to harbor SIVcpz, and their viruses form divergent subspecies-specific phylogenetic lineages (SIVcpzPtt and SIVcpzPts) (4). HIV-1 is most closely related to SIVcpzPtt (5), but this virus has been detected only rarely and then only in captive apes (1, 5–7). There is no counterpart of SIVcpzPts that is known to infect humans (4, 8–10).

Wild-living chimpanzees are reclusive and highly endangered and live in remote jungle areas. To study chimpanzees in their natural habitat, we developed methods to detect SIVcpz-specific antibodies and nucleic acids in fecal samples collected from the forest floor (9–11). In addition, we developed genotyping approaches to amplify host mitochondrial and genomic markers (polymorphic microsatellite loci) from these same specimens for species, gender, and individual identification (11, 12).

These methods were validated in captive and habituated apes of known infection status (13). We used these noninvasive approaches to conduct the first molecular epidemiological field study of SIVcpz in wild-living nonhabituated chimpanzees in west central Africa.

Cameroon is home to two chimpanzee subspecies, P. t. vellerosus in the north and P. t. troglodytes in the south, with the Sanaga River forming the boundary between their ranges (Fig. 1). In the present study, we collected 599 fecal specimens at 10 forest sites throughout the southern part of Cameroon (Fig. 1). All field sites, except one (WE), were in the range of the P. t. troglodytes subspecies. To establish the species and subspecies origin of each sample, a 498-base pair (bp) mitochondrial DNA (mtDNA) (D-loop) fragment was amplified from fecal DNA and subjected to phylogenetic analysis (13). Eighty-six specimens were degraded, and 67 samples contained gorilla mtDNA sequences (table S1). The remaining 446 samples were of chimpanzee origin: 423 from P. t. troglodytes and 23 from P. t. vellerosus. These comprised 82 unique mtDNA haplotypes (fig. S1 and table S2). Consistent with the recognized ranges of the two subspecies, all 23 P. t. vellerosus speci-

Fig. 1. Natural ranges of the four chimpanzee subspecies (top) and locations of wild chimpanzee study sites WE, MT, DG, DP, BQ, EK, CP, BB, MB, and LB in southern Cameroon (inset and bottom). Field sites with endemic SIVcpzPtt infection are color-coded to correspond with the SIVcpzPtt lineages shown in Figs. 3 and 4.
mens were collected north of the Sanaga River, whereas 421 of 423 P. t. troglodytes samples were collected south of the river (table S1).

All mtDNA-positive fecal samples were tested for virus-specific antibodies with a sensitive immunoblot assay specifically developed for surveys at remote field sites (13). This analysis identified 34 specimens, all from P. t. troglodytes apes, that contained antibodies reactive with HIV-1 antigens (Fig. 2). Twelve samples exhibited a strong and broadly cross-reactive Western blot profile that was virtually indistinguishable from the HIV-1–positive human plasma control. Eighteen additional samples reacted with both the HIV-1 envelope (gp160) and major core (p24) proteins, thus also meeting formal criteria for HIV-1/SIVcpz antibody positivity. Four samples (EK502, EK506, MB245, and MB248) reacted only faintly with a single HIV-1 protein (p24) and were classified as indeterminant. None of 23 P. t. vellerosus or 67 gorilla specimens exhibited detectable Western blot reactivity to any HIV-1 protein (table S1).

To corroborate the fecal antibody results, RNA was extracted from all immunoblot-reactive samples and subjected to reverse transcription polymerase chain reaction amplification using consensus env and pol primers. In addition, fecal DNA was used to amplify polymorphic microsatellite loci to identify and distinguish individual apes and to amplify a portion of the amelogenin gene for gender determination (13). These analyses revealed that the 34 immunoblot-reactive samples represented 16 different P. t. troglodytes apes (7 males and 9 females). Each of these apes had detectable virion RNA in one or more fecal samples (table S3). SIVcpz env (~390 bp) and/or pol (~890 bp) sequences were amplified from 31 of 34 (91%) immunoblot-reactive samples, including all four specimens with indeterminant Western blot reactivity (Fig. 3 and table S3). These data,
together with previous findings for SIVcpzPsinfected apes (10), indicate that fecal antibody reactivity to a single HIV-1 Gag protein is indicative of SIVcpzPtt infection (14).

The prevalence of SIVcpzPtt infection in wild chimpanzee communities was estimated for each of the 10 field sites (table S1). For the DP, EK, MB, BB, and LB communities, this was done based on the proportion of infected individuals as determined by microsatellite analyses, taking into consideration assay sensitivities and specimen degradation (tables S1 and S4). For the remaining sites, prevalence rates were estimated based on the proportion of antibody- and/or SIVcpz virion RNA-positive fecal samples, while also adjusting for repeat sampling (13). The results indicated widespread but notably uneven SIVcpzPtt infection of wild-living *P. t. troglodytes* apes, with prevalence rates ranging from 23 to 35% in the LB, EK, and MB communities; 4 to 5% in the DP and MT communities; and the absence of infection in the WE, DG, BQ, BB, and CP communities.

To determine the evolutionary relationships of the 16 new SIVcpzPtt viruses to each other and to previously characterized SIVcpz and HIV-1 strains, *pol* and *env* sequences were subjected to phylogenetic analyses. All of the newly identified SIVcpz strains were found to fall within the HIV-1 group N lineages (Fig. 3). The new viruses reduced the lengths of the branches marking the cross-species transmission events for all genomic regions by almost half (arrows in Fig. 4). Given these short branch lengths, it is highly unlikely that other SIVcpzPtt strains exist that are significantly more closely related to HIV-1 groups M and N than are the viruses from the MB/LB and EK communities. Indeed, expanded field studies in southern Cameroon by our group have identified additional SIVcpzPtt strains, including nine from the MB/LB area, whose sequences support this conclusion and corroborate the phylogenetic relationships shown in Figs. 3 and 4 (15).

The findings presented here, together with prior studies, provide for the first time a clear picture of the origin of present-day human AIDS viruses to distinct chimpanzee communities. In subgenomic *pol* and *env* regions, SIVcpzPtt strains from the MB/LB and EK sites were much more closely related to HIV-1 groups M and N, respectively, than were any previously identified SIVcpz strains (Fig. 3). Full-length genome analysis of 4 of the 16 new viruses confirmed and extended these findings, revealing strong statistical support for the clustering of HIV-1 groups M and N with the MB/LB and EK lineages of SIVcpzPtt, respectively (Fig. 4). Moreover, inclusion of the new viruses reduced the lengths of the branches marking the cross-species transmission events for all genomic regions by almost half (arrows in Fig. 4). Given these short branch lengths, it is highly unlikely that other SIVcpzPtt strains exist that are significantly more closely related to HIV-1 groups M and N than are the viruses from the MB/LB and EK communities. Indeed, expanded field studies in southern Cameroon by our group have identified additional SIVcpzPtt strains, including nine from the MB/LB area, whose sequences support this conclusion and corroborate the phylogenetic relationships shown in Figs. 3 and 4 (15).

Thus, an extensive set of molecular epidemiological data all points to chimpanzees in southeastern and south central Cameroon as the sources of HIV-1 groups M and N, respectively.

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Mast Cells Can Enhance Resistance to Snake and Honeybee Venoms

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Snake or honeybee envenomation can cause substantial morbidity and mortality, and it has been proposed that the activation of mast cells by snake or insect venoms can contribute to these effects. We show, in contrast, that mast cells can significantly reduce snake-venom–induced pathology in mice, at least in part by releasing carboxypeptidase A and possibly other proteases, which can enhance resistance to the morbidity and mortality induced by animal venoms. This finding identifies a new biological function for mast cells in enhancing resistance to the morbidity and mortality induced by animal venoms.

Venomous reptiles and their prey have coexisted for ~200 million years (1), and snake envenomation still accounts for considerable human morbidity and mortality worldwide (2, 3) (SOM Text 1). The mechanisms by which snake envenomation can produce tissue injury and death have been studied extensively (3–5), and it is known that many components of snake venoms can induce problematic for HIV diagnostics and vaccines. The present report describes molecular tools and noninvasive strategies that can be used to explore these possibilities as well as the molecular ecology of pathogens in endangered species more generally.

References and Notes
13. Materials and methods are available as supporting material on Science Online.
14. In contrast to plasma samples from uninfected humans, which exhibit false positive Western blot reactivity to HIV-1 p24 in as many as 10 to 15% of individuals (www.lda.gov/cher/products/hiv1cam052898.htm), we have found no such nonspecific cross-reactivity of chimpanzee immunoglobulin extracted by the RINAloter method from over 2000 fecal specimens.

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