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A new human immunodeficiency virus derived from gorillas

Jean-Christophe Plantier[1], Marie Leoz[1], Jonathan E Dickerson[2], Fabienne De Oliveira[1], François Cordonnier[3], Véronique Lemée[1], Florence Damond[4], David L Robertson[2] & François Simon[5]

We have identified a new human immunodeficiency virus in a Cameroonian woman. It is closely related to gorilla simian immunodeficiency virus (SIVgor) and shows no evidence of recombination with other HIV-1 lineages. This new virus seems to be the prototype of a new HIV-1 lineage that is distinct from HIV-1 groups M, N and O. We propose to designate it HIV-1 group P.

HIV-1, the virus principally responsible for the AIDS pandemic, arose through cross-species transmission of a retrovirus (SIVcpz*Ptt*) found in chimpanzees (*Pan troglodytes troglodytes* (*Ptt*))[1,2]. Another SIV (SIVgor), recently discovered in wild-living gorillas (*Gorilla gorilla gorilla*)[3], has many of the biological properties necessary for human infection[4]. We have now identified a new human immunodeficiency virus closely related to SIVgor in a Cameroonian woman. This new HIV-1 variant is distinct from the three established groups of HIV-1, namely M (major or main), N (non-M, non-O) and O (outlier)[5,6].

Since 2001, a French network of reference laboratories has been monitoring HIV genetic diversity. Infection with an unusual variant is suspected when RNA viral load assays or molecular tests are negative in an individual with acquired immunodeficiency naive of antiretroviral therapy. As part of these surveillance activities, we analyzed serial samples from a 62-year-old woman (subject number RBF168) who was found to be HIV seropositive in 2004, shortly after moving to Paris from Cameroon (**Supplementary Methods**). Several HIV-1 screening tests were all reactive, and western blotting with HIV-1 group M proteins showed weak reactivity against the envelope glycoprotein 120 and no reactivity against Gag p18 protein (**Supplementary Methods** and **Supplementary Fig. 1**). She currently has no signs of AIDS, remains untreated and has a stable $CD4^+$ cell count of about 300 cells per mm^3 (**Supplementary Fig. 2**). Her viral load has been consistently high since diagnosis (4.4 to 5.3 log copies per ml) in nonspecific group M and O PCR commercial assays (LCx HIV RNA Quantitative and Real*Time* HIV1, Abbott) and in an in-house real-time RT-PCR assay[7] (**Supplementary Fig. 2**). The virus replicates in cultured human donor peripheral blood mononuclear

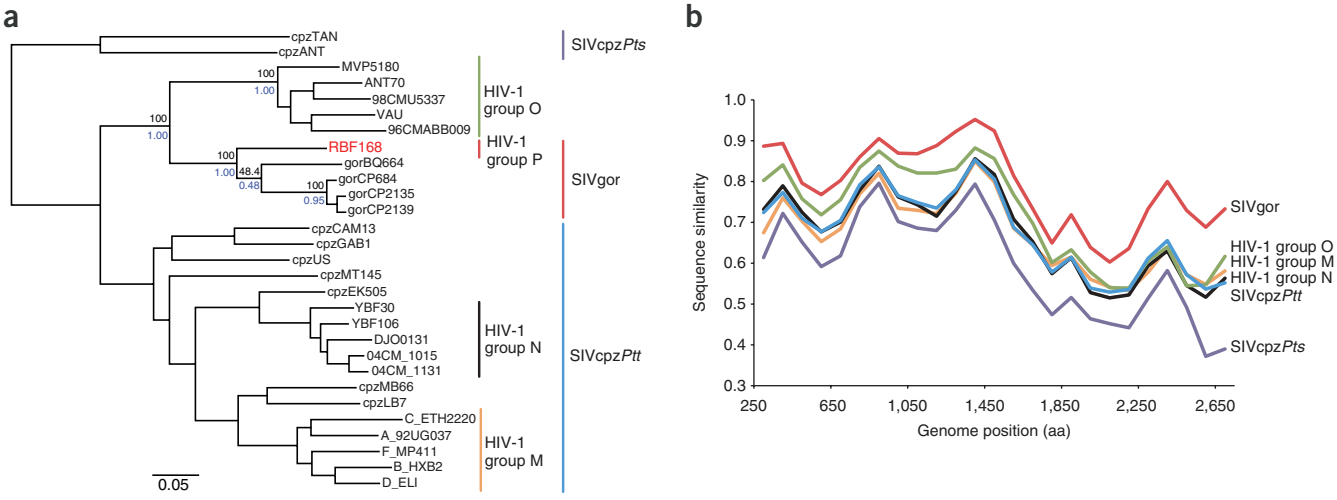

Figure 1 Evolutionary relationship of strain RBF168 to HIV-1, SIVcpz and SIVgor. (**a**) Maximum likelihood phylogeny inferred from concatenated amino acid alignments corresponding to the partial sequences available for SIVgorBQ664 (ref. 4); 1,052 amino acid positions remained after stripping gap-containing sites. The support values (indicated for key nodes only) in black above the branches are from 1,000 maximum likelihood bootstraps (shown as percentages), whereas posterior probabilities from amino acid Bayesian analysis are shown in blue below the branches (shown as proportions). (**b**) Average sequence similarity (250 amino acid windows, 100–amino-acid increments) of RBF168 with representative strains of HIV-1 groups M, N and O, SIVgor, SIVcpz from *Pan troglodytes schweinfurthii* (SIVcpz*Pts*) and SIVcpz*Ptt* across the concatenated translated gene sequence alignments. Similar results were obtained with the nucleotide sequence alignment (data not shown).

[1]Laboratoire associé au Centre National de Référence du Virus de l'Immunodéficience Humaine, Centre Hospitalier Universitaire de Rouen, Equipe d'Accueil EA2656, Faculté de Médecine-Pharmacie, Université de Rouen, France. [2]Faculty of Life Sciences, University of Manchester, UK. [3]Hôpital Louis Mourier, Colombes, France. [4]Hôpital Bichat, Paris, France. [5]Hôpital Saint-Louis, Institut National de la Santé et de la Recherche Médicale U941, Faculté de Médecine, Université Paris-Diderot, Paris, France. Correspondence should be addressed to J.-C.P. (jean-christophe.plantier@univ-rouen.fr).

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cells and is easily isolated from both the subject's plasma and peripheral blood mononuclear cells (**Supplementary Methods**). Her viral load cannot, however, be quantified with a group M–specific commercial assay (Amplicor Monitor v1.5, Roche) or with an academic assay (Generic HIV charge virale, Biocentric[8]) (**Supplementary Fig. 2**). We did not obtain amplification with complementary group M–specific PCRs (**Supplementary Methods**). We initially suspected HIV-1 group O infection, endemic in western central Africa, especially in view of the subject's Cameroonian origin. However, amplification with our usual group O primers failed (**Supplementary Methods** and **Supplementary Table 1**), leading us to search for a divergent virus by using a nonspecific extra-long RT-PCR method. We successfully amplified the viral genome with this approach, allowing us to fully sequence it (**Supplementary Methods**, **Supplementary Fig. 5** and **Supplementary Table 2**).

Evolutionary analysis of the near-complete genome sequence (**Supplementary Methods**) shows that the RBF168 strain is most closely related to SIVgor (**Fig. 1a** and **Supplementary Fig. 3**), and similarity plotting confirms that this relationship is maintained in all regions of the genome (**Fig. 1b**). Before the discovery of strain RBF168, HIV-1 group O was the lineage most closely related to SIVgor, but it is too divergent to be directly derived from current SIVgor strains[4]. As strain RBF168 clusters significantly with SIVgor strains (see support values on tree, **Fig. 1a** and **Supplementary Fig. 3**), the most likely explanation for its emergence is gorilla-to-human transmission of SIVgor (**Supplementary Fig. 4a**,**b**). Similar to the proposed chimpanzee origin for the HIV-1 group O and SIVgor lineage[4], we cannot rule out the possibility that SIVcpz gave rise to strain RBF168, either indirectly by transmission to gorillas and then to humans (**Supplementary Fig. 4a**,**b**) or directly by transmission to humans and also to gorillas (**Supplementary Fig. 4c**). Detection of RBF168-like viruses in chimpanzees would be needed to confirm this possibility.

Strain RBF168 thus represents a new HIV-1 variant and is the prototype of a new human lineage that we designate as putative group P, pending the identification of further human cases, in keeping with nomenclature guidelines[6]. The human case described here does not seem to be an isolated incident, as before coming to Paris the subject had lived in the semiurban area of Yaoundé, the capital of Cameroon, and reported no contact with apes or bush meat (**Supplementary Methods**), and the variant's high level of replication *in vivo* and ready isolation in culture indicate that it is adapted to human cells. This efficient replication of RBF168 is rather unexpected, given the absence of an arginine (or lysine) at position 30 in the Gag protein, considered a signature of human-specific adaptation of HIV-1 (ref. 9). Contrary to most HIV-1 strains (apart from group M subtype C), but like SIVgor and all SIVcpz*Ptt* strains[9], RBF168 has a methionine at this amino acid position.

The human prevalence of this new lineage remains to be determined. Strain RBF168 shows typical HIV-1 behavior in serological and nonspecific molecular tests, suggesting that it could be circulating unnoticed in Cameroon or elsewhere. HIV screening tests and molecular tools have improved markedly over the past two decades, enabling the distinct HIV types and groups to be detected. This increased sensitivity, however, may paradoxically mask the circulation of divergent strains. Indeed, new variant infections can now be detected only by monitoring discrepancies between immunological status and virological results in molecular assays. Currently, there is no simple detection algorithm based on existing serological and molecular tools, and, therefore, only nucleotide sequencing can identify further HIV-1 group P strains.

In conclusion, our findings indicate that gorillas, in addition to chimpanzees, are likely sources of HIV-1. The discovery of this novel HIV-1 lineage highlights the continuing need to watch closely for the emergence of new HIV variants, particularly in western central Africa, the origin of all existing HIV-1 groups.

Accession codes. The near full-length sequence of strain RBF168 has been submitted to GenBank under accession number GQ328744.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

J.-C.P., M.L. and F.D.O. conceived of and designed the experiments. M.L., F.D.O. and V.L. performed the molecular and serological experiments. J.E.D. and D.L.R. performed the computational analysis. F.C. managed the subject and collected epidemiological data. J.-C.P., V.L. and F.D. monitored the subject's virologic status. J.-C.P, M.L., J.E.D., F.D.O., D.L.R. and F.S. wrote the paper.

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