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HIV-1 Group O Origin, Evolution, Pathogenesis, and Treatment: Unraveling the Complexity of an Outlier 25 Years Later

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Abstract

Twenty-five years ago, an aberrant HIV-1 (now classified as HIV-1 group O) was described from a Cameroonian HIV patient living in Belgium. The epicenter of group O was later found to be in Central Africa, overlapping with the geographical location of the central chimpanzees* (Pan troglodytes troglodytes) *and western gorillas* (Gorilla gorilla)*, the likely original hosts of group O. Although the prevalence of group O has remained low at 1-2% in Cameroon, some European countries (France, Spain, Belgium) with strong colonial ties to Central Africa have reported the highest prevalence out of Africa. The sequence diversity between HIV-1 group O and M strains is huge, reaching 50 and 30% in the envelope and pol, respectively. This diversity has hindered diagnosis, monitoring, and treatment of group O-infected patients. Due to the intrinsic presence of the C181 mutation in group O, more than 60% of the approximately 30,000 individuals that live with this virus are faced with the challenge of drug resistance to some currently used antiretroviral therapies, notably the non-nucleoside reverse transcriptase inhibitors. Despite its susceptibility to most antiretroviral therapies, some group Os show a high variable baseline susceptibility to enfuvirtide (T20) and maraviroc. Group O strains are the least fit among all HIV-1 and -2 and restrict tetherin using their Nef but not Vpu as reported for group M. Although limited follow-up studies indicate that the natural course of group O is similar to that of M, these viruses are dominantly CCR5 tropic even late in infection, suggesting slow disease progression. This review summarizes important findings that marked the discovery, origin, spread, evolution, pathogenesis, and treatment of group O within the last 25 years. **(AIDS Rev. 2015;17:147-58)**

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Key words

HIV-1 group O. Evolution. Pathogenesis. Treatment.

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Introduction

The human immunodeficiency virus type 1 (HIV-1) was first isolated in 1983 and is comprised of four different groups, namely M-major, O-outlier, N-non, M-non-O, and P, which were independently transmitted to humans from nonhuman primates infected with simian immunodeficiency virus (SIV) (Fig. 1). Specifically, groups M and N jumped from the chimpanzees *Pan troglodytes troglodytes, (Ptt)* in Southern Cameroon[1], while groups O and P are closely related to SIVgor isolated from western lowland gorillas *(Gorilla gorilla)* in the same geographical region[2-4]. Group P is the closest relative to SIVgor (Fig. 1), an SIV that likely derived from SIVcpz some 100-200 years ago[3-5]. All four HIV-1 groups are phylogenetically closely related to SIVcpzPtt strains from central chimpanzees. Among the four HIV-1 groups, only M has established a strong hold within the human population, causing more than 98% of all HIV infections globally[6]. Groups N, O, and P have been reported albeit rarely and have their highest prevalence (~ 1-2% of all HIV infections) in West and Central Africa, with Cameroon as the epicenter (Fig. 2 A and 2 B)[5,7-9]. Combined, groups N and P have been reported in less than 25 individuals, mostly of Cameroonian origin[5,7]. Group O is therefore the most prevalent among these three rare variants and it is estimated that about 100,000 people have been infected with this group since the start of the HIV epidemic[4,10].

The first case of group O infection was described in 1990 when an HIV strain known as ANT70 from a Cameroonian patient living in Belgium was isolated and partially characterized (Table 1)[11]. Four years later in 1994, this strain, as well as two other prototypic strains, MVP5180 and VAU, isolated from Cameroonian and French patients, respectively, was completely characterized (Table 1)[12-14]. Sequences from these three strains were found to be as distant apart from each other as reported for group M subtypes (Figs. 1 and 2).

Although the genetic diversity of group O strains has been ascertained, it is still unclear what role this diversity has played in shaping their limited spread, pathogenesis, and treatment. This review summarizes published findings on group O strains and sheds more light on its evolution and spread within the last 25 years. We also examine how recent knowledge of human restriction factors, fitness, and therapy has helped us to understand why this virus group has been limited in spread and resistant to certain antiviral drugs.

Origin, evolution, and geographical distribution

Although first reported 25 years ago, HIV-1 group O has been circulating in the human population for much longer and might have gone unnoticed as a result of its diversity (Table 1, Fig. 1). Molecular clock estimates date the time to the most recent common ancestor (tMRCA) of group O to the 1920s (1890-1940) or 1930s (1914-1944), which is similar to estimated tMRCA of group M of 1908 (1884-1924) (Fig. 1)[15-17]. Using a two-phase exponential logistic model, Faria, et al, determined the group O exponential growth rate during the early phase of the epidemic between 1920 and 1960 to be 0.07 per year, slightly lower than the 0.1 per year observed for group M[18]. This study proposed that, after 1960, the exponential growth of group M was much faster, doubling to 0.27 per year, while that of group O remained stable[18]. However, a recent report indicated that group O had two phases of exponential growth; the first during 1940-1960 and the second between 1970-1990[17]. The earliest reported case of a group O infection was out of Africa, in the family of a Norwegian sailor who had visited seaports in West and Central Africa on several occasions in the 1960s (Fig. 2 A and 2 B). This sailor is believed to have been infected with HIV between 1961-65, but only showed symptoms in 1966[19]. His daughter (T29, Fig. 2 C) was born in 1967, fell sick in 1969, and died of AIDS in 1976. Phylogenetic analyses of partial integrase-vif sequences of patient T29 classified this strain as Clade B (Fig. 2 B). Similarly, the third group O isolate (VAU), described in the 1990s, was from a French patient who had never travelled to Africa and died of AIDS in 1992 (Fig. 2 C). These two cases suggest that HIV-1 group O had been circulating and transmitted in Europe long before its isolation in 1990[10,14,19]. In Europe, France has reported at least 136 cases since 1992, with a prevalence of 0.1% among newly diagnosed subjects, the highest out of Cameroon[10]. This is not surprising given the historical relationship between France and Cameroon in particular and Francophone Africa in general. In Germany, a single case of Cameroonian origin was reported in the 1990s[20]. Cases in Spain have been linked with travels to Equatorial Guinea and Cameroon, both Central African countries (Fig. 2 A)[21]. In the USA, the two reported cases involved immigrants from Cameroon[22,23]. Quite clearly, group O infections in Europe and the USA have been limited within family circles at best, with limited transmission to the general public.

In West and Central Africa, group O is dominant in three countries (Cameroon, Gabon and Equatorial Guinea) with

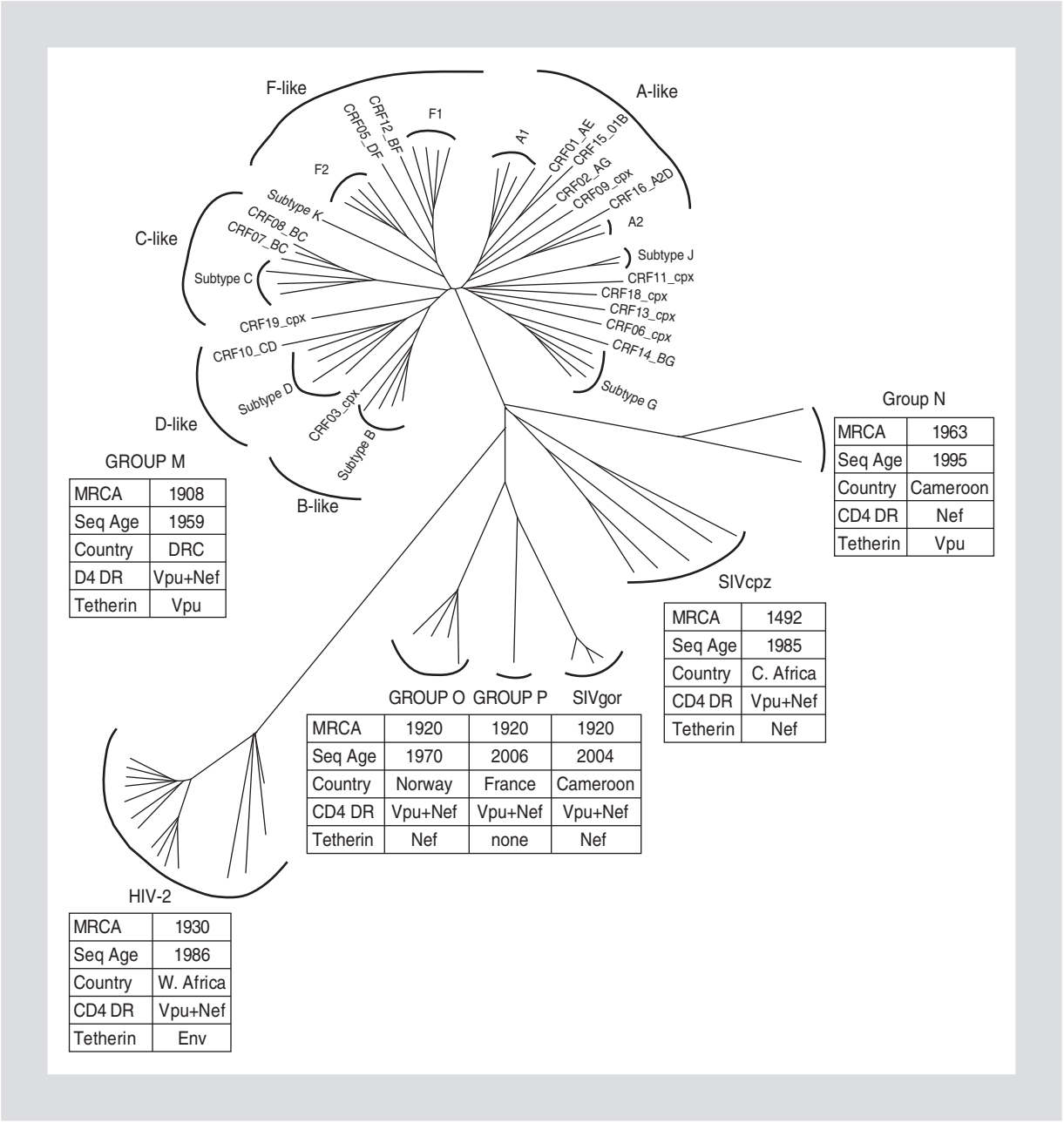

Figure 1. *Phylogenetic classification of HIV-1 (groups M, N, O and P), HIV-2 and SIV (SIVcpz and SIVgor) relating to their origin, evolution and spread.*
The inserted tables show the time to the most recent common ancestor (MRCA), oldest available sequence (Seq Age), country or location of oldest sequence (Country); CD4 down-regulation (CD4 DR) by Nef and/or Vpu; and tetherin antagonism (Tetherin) by Nef, Vpu or Env.

a reduced prevalence moving westwards (Fig. 2 A). The location of Cameroon (especially the southern portion) puts it at the center of the geographical range of the *Pan troglodytes troglodytes* chimpanzee sub-species that harbors SIVcpz, the ancestors of HIV-1, as well as western lowland gorillas *(Gorilla gorilla),* the host of groups P and O[2,4]. Alongside group O, groups N and P are also endemic to Cameroon[5,24-27]. Group O prevalence in Cameroon has been decreasing steadily since the beginning of the epidemic (Fig. 2 B)[28]. From 1989-1998, the prevalence reduced from 8.6 to 1.4% among HIV-1 infected patients[28,29]. Interestingly, 20% of samples collected between 1986-1988 in this survey were reactive to group O variable loop 3 (V3) peptides[28]. Independent serologic screening of 3,500 specimens collected between 1998-2000 from three regions in northwestern

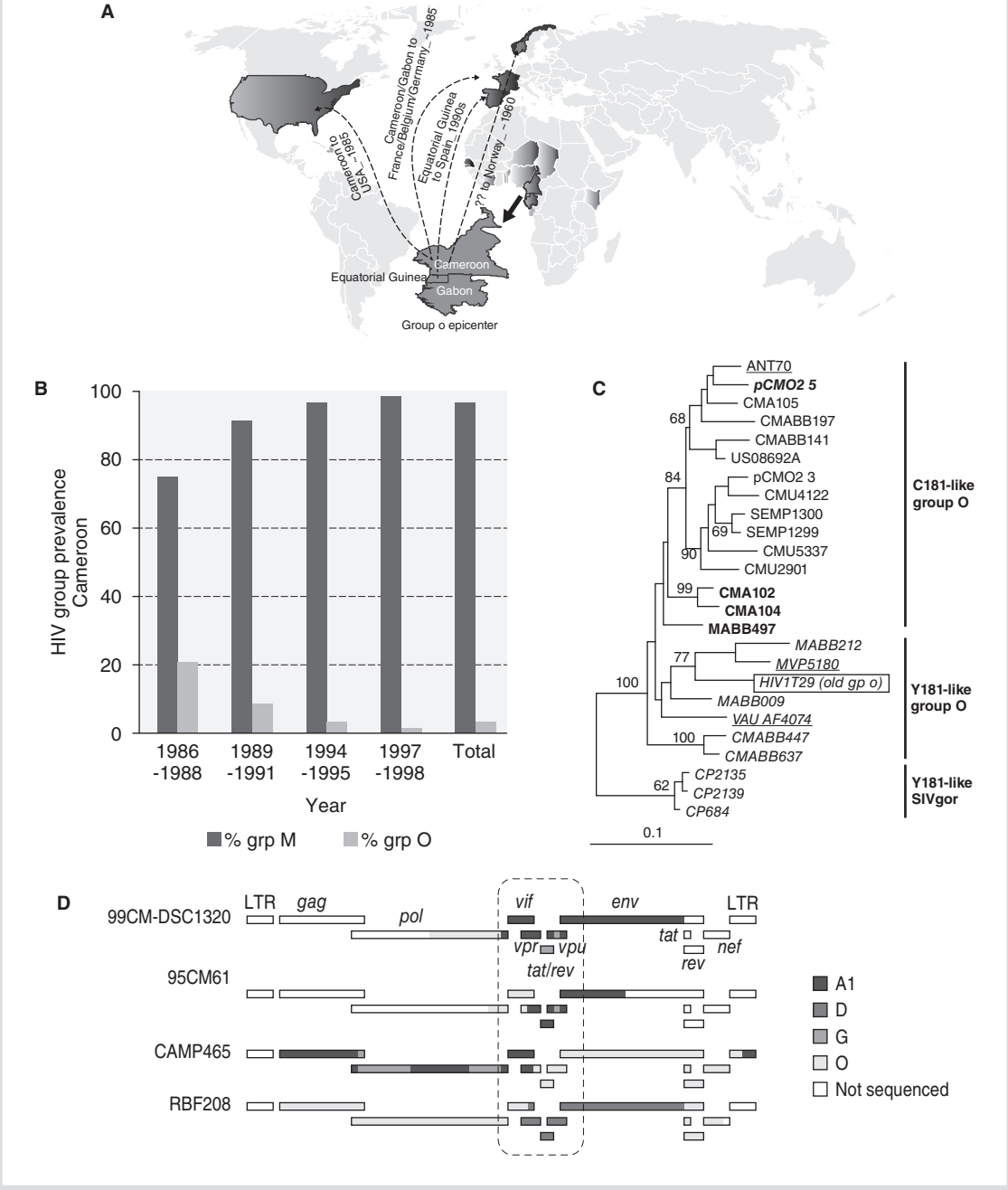

Figure 2. *HIV-1 group O global distribution, spread and genotypes.* ***A:*** *HIV-1 group O origin, spread and global distribution; doted arrows represent the likely source of infections during the dates inscribed on them. The country of origin of the Norwegian group O is unknown and is represented as "??". The countries within the epicenter of group O (Cameroon, Equatorial Guinea and Gabon) are shown in black and enlarged insert. Other countries (Senegal, Belgium, France, Belgium, Germany, Norway and United States) where full-length genomic sequences or more detailed information about group O strains are known are also shown in black; Countries in dark grey have reported antibodies reactive to group O ELISAs.* ***B:*** *Evolution of group O prevalence in Cameroon from 1986-1998. Figure was generated based on published prevalence data[28].* ***C:*** *Genotypic classification of group O; Phylogenetic analyses of integrase-vif sequences. The ancient group O sequence T29 isolated from a 1976 specimen (boxed) is from the daughter of the index patient in Norway. The three prototypic O isolates ANT70, MVP5180 and VAU are underlined.* ***D:*** *Genomic structure of O/M recombinants; the 4 partial or near-complete genomic structures of published O/M recombinants are shown. The boxed area shows the accessory genes where recombination is frequent. The first three genomes (95CM61, 99CM-DSC1320, CAMP645) resulted from dual or multiple infections of groups O and M leading to recombination in the patient from whom the samples were collected. RBF208 was likely transmitted as a recombinant.*

Table 1. Important milestones in the global evolution and understanding of HIV-1 group O

Year	Event/Finding/Report	Reference
1990	First report of an aberrant HIV-1 strain	De Leys, et al. 1990
1994	Detailed characterization of two reference group O viruses	Gürtler, et al. 1994 Vanden Haesevelde, et al. 1994
1996	First report of a dual O/M HIV infection in a patient from Benin (West Africa)	Heyndrickx, et al. 2006
1997	Ancient group O (1970s) from frozen Norwegian samples Some group O strains are naturally resistant to NNRTIs HIV-1 group O antibodies identified in 8 countries in West and Central Africa First report of a triple clade group M/O infection in a single subject in Cameroon	Jonassen, et al. 1997 Descamps, et al. 1997 Peeters, et al. 1997 Takehisa, et al. 1997
1999	Description of group M/O recombinant viruses in unlinked patients	Peeters, et al.; Takehisa, et al. 1999
2004	HIV-1 group O time to most recent common ancestor estimated to be 1920	Lemey, et al. 2004
2005	Generation and characterization of the first group O infectious molecular clone	Tebit, et al. 2005
2006	Group O demonstrates lowest replicative fitness among HIV-1 and HIV-2 strains	Arien, et al. 2006
2006	HIV-1 group O-like sequences found among gorillas in southeastern Cameroon	van Heuverswyn, et al. 2006
2009	Identification of "gorilla-like" group O sequences in humans and named "group P"	Plantier, et al. 2009
2011	Tetherin played a major role in the limited spread of HIV-1 group O	Sauter, et al. 2010
2014	HIV-1 group O Nef proteins antagonize human tetherin	Kluge, et al. 2014
2015	Lowland gorillas are the source of HIV-1 groups P and O The two-phase emergence of non-pandemic HIV-1 group O in Cameroon	D'Arc, et al. 2015 Leoz, et al 2015

NNRTI: nonnucleoside reverse transcriptase inhibitor.

Cameroon confirmed a group O prevalence of 0.4%, lower than the 1% in the northern region and 6% in the central and southern parts of Cameroon, the latter two located closer to the equatorial rain forest[30]. In general, group O prevalence in Cameroon has been stable at 1-2% during the last decade, while group M prevalence increased steadily from the late 1980s to the late 1990s (Fig. 2 B). Nigeria, Cameroon's neighbor to the west, identified its first case of group O in the Cross River State, which borders the southwest region of Cameroon[31]. A recent survey in Gabon showed a decrease in group O prevalence from 0.9% in 1997 to 0.4% in 2011[32]. In all, 10 West and East African countries, including Togo, Senegal, Niger, Ivory Coast, Benin, and Kenya, have reported sporadic cases of group O, with prevalences ranging from 0.07% in Senegal to 2.1% in Cameroon (Figs. 1 and 2 A)[33-37]. Interestingly, despite a high diversity of HIV-1 and the presence of ancient group M strains from the Democratic Republic of Congo (DRC), no case of group O has been reported from this country[38,39]. Similarly, no group O cases have been found in Central and South America, Asia, or Eastern Europe (Fig. 2 A).

Genetic diversity, dual infection, and recombination

It was almost a decade after its discovery that genuine attempts were made at classifying group O strains into subtypes similar to what was obtained for group M. Classification was made difficult partly by the lack of group O sequences and its high diversity. Sequence difference between the envelope and pol of the prototypic group O (ANT70 and MVP5180) and group M subtype B (M-B) strains were reported to be 50 and 27%, respectively[13]. This difference was also reflected in entire genome analyses, showing a homology of about 50% to group M-B[13]. Earlier phylogenetic analyses of the env gene of the prototypic group O strains HIV-1_{ant70}, HIV-$1_{mvp5180}$ and HIV-1_{vau} indicated that these viruses were genetically separated, with distances similar to observations between group M subtypes[14]. Analyses of shorter fragments in gag (p24) and env (C2-V3) of group O isolates showed limited clustering patterns with no clear phylogenetic grouping[40-43]. Later studies involving longer sequence fragments and the complete sequences of

pol and envelope classified group O isolates into two clades (O:A and O:B)[44], or three clades (O:A, O:B and O:C)[45], or five clades (I, II, II, IV, V)[46]. Recently, a more detailed phylogenetic analysis of 190 group O sequences suggested a comet-like tree topology with two major clusters H (comet head) and T (comet tail), the former having three sub-clusters (H1, H2, H3). Mostly, clade O:A (ANT70-like, C181 or H strains) is the most dominant clade and exhibits the greatest variability[17,45].

An in-depth characterization of group O pol identified predictable clustering patterns based on the presence of a cysteine (C) or tyrosine (Y) at position 181 of the reverse transcriptase (RT) enzyme (C181 and Y181 respectively; Figs. 2 C and 3)[47]. In these classification systems, clade O-A or Ia/Ib/IV clusters with the C181 lineage (ANT70-like). Using a larger set of sequences, Leoz, et al. recently confirmed a strong association of the "H strains" with position C181 of RT, although this position was found not to be an adequate marker for classification[17]. Importantly, C181 coexists as a natural polymorphism in group O, unlike in group M where Y181 is the only naturally occurring genotype. With the exception of C181 in group O RT, a large collection of SIV sequences from chimpanzees (SIVcpz), gorilla (SIVgor), red-capped mangabeys (SIVrcm), drill (SIVdrl), and mandrills (SIVmnd) are phylogenetically linked to the Y181 lineage[47]. The C181 variants cause resistance to certain non-nucleoside reverse transcriptase inhibitors (NNRTI), such as nevirapine and etravirine (discussed later), and comprise more than 60% of all group O strains characterized to date[10,47]. Notably, the most ancient group O sequences from Norway cluster with the less-dominant Y181-like Clade B (Fig. 2 C), indicating that Y181-clade viruses were already present in West Central Africa as far back as 1960[17,19]. To date, no simian progenitor of the larger C181 lineage of group O has been reported[3].

Earlier in the epidemic, full-length sequences of group O strains were limited to the prototype strains ANT70 and MVP5180. Currently there are 15 full-length group O sequences available in the HIV database, one of which is an infectious molecular clone that has enabled group O functional characterization[47-49]. Specifically, this clone was important in delineating the role of N-linked myristoylation as a requirement for associating HIV-1 group O Nef with cellular membranes, a function similar to that of group M Nef[48].

There is a clear discrimination in the accessory genes and protein sequences of group O and M strains, which may have influenced their evolution. Among the four accessory genes (vif, vpr, tat, vpu) the vpu is the most diverse, with a mean divergence of 16 and 21% within group O and M, respectively. Vif, vpr, and tat are more conserved in both groups, with divergence ranging from 9-13%[50]. At the protein level, mean divergence of 26.3% (range, 7.8-44%) is also observed among group O. Most prominent is a four amino acid insertion at the alpha-helix-1 of the N-terminal of the cytoplasmic tail important for vpu localization in the endoplasmic domain[50,51]. As discussed below, the vpu of group O might have influenced its evolution and spread in the present HIV pandemic.

Recombination and the generation of mosaic SIV/HIV strains have been crucial in HIV's evolution[52]. Interestingly, the recently described SIVgor strain (BQID2), which is most closely related to group O, is a mosaic of group O and SIVgor[4]. Within the last 15 years, more than a dozen cases of group O/M dual infections have been reported[53-57]. Recent reports suggest that about 9% of all group O infections in Cameroon involves O/M dual infection[57]. Five O/M recombinants have been found co-circulating in the same patient, with both their parental O and M strains (n = 3) or with only the group O strain (n = 2)[54,58]. The earliest O and M infections were reported in 1996 and 1997, respectively, in patients from Benin and Cameroon[36]. The latter involved a triple-clade infection (single group O clade and two group M clades) in one subject (Table 1)[53]. Significantly, three O/M recombinants (99CM-DSC1320, 95CM61, CAMP485) with distinct group O-A, O-B or M-A, M-G genomic composition and crossover points have emerged from dually infected subjects (Fig. 2 D)[54-56,58]. A fourth O/M recombinant involving M-D was recently reported (Fig. 2 D)[57]. The subtype crossover points are mostly located within the accessory genes vif, vpr, or vpu, but also occur in the integrase (99CM-DSC1320) or long terminal repeat (CAMP645), creating a heterologous TAR-M/Tat-O pair (Fig. 2 D).

While some of these recombinants coexisted with their parental subtypes (dual/multiple infection) in the same patient, others existed alone, indicating that they were transmitted as recombinants from other subjects. Higher fitness of recombinants relative to their parental strains is instrumental in determining the dominant quasispecies[54]. The dominance of group M envelopes in O/M recombinants (Fig. 2 D) might suggest a better entry efficiency of group M envelopes compared to group O. Intragroup O recombination between clades A and B have also been reported, although rarely[45,59].

HIV-1 group O diagnosis, mother-to-child transmission, and pathogenesis

Current diagnostic ELISA tests include antigens that allow the detection of group O specific antibodies.

However, some assays might still generate false-negative results, considering group O's genetic diversity. Confirmatory assays for group O are still unreliable as detection is based on interpretation of varying profiles of weak and/or non-reactive Gag, Pol, and Env proteins on group M-based Western blots. Serological reactivity is best confirmed using group O-specific gp120 V3 loop or gp41 immunodominant peptide ELISA[56,60]. Molecular assays for the quantification of group O viral load have significantly improved in sensitivity within the last decade, but are still not optimal.

Despite the limited number of reported group O mother-to-child transmission (MTCT) cases in the last 25 years, it is interesting to note that the first two cases of HIV-1 group O reported in Norway and France also represent the earliest documented cases of group O MTCT[14,19]. The first case of group O MTCT in Cameroon was reported in 1998[61]. Follow-up of the child's virus for 30 months showed that one of two variants present in the mother was selectively transmitted to the child. This variant increased in nucleotide sequence diversity with time from 0.8 to 6%, and a novel genotype appeared after 30 months. The non-synonymous/synonymous mutation ratio also increased with age, from 0.75 at birth to 1.86 at 30 months, similar to reports in group M[61,62].

Relative to group M, the pathogenic course of group O remains largely understudied. Typically during disease progression, individuals infected with group M strains develop neutralization escape variants and switch tropism from the infecting CCR5 (R5) to CXCR4 (X4) or dual (R5X4) tropic viruses[63]. The appearance of X4 or dual-tropic strains is accompanied by the depletion of $CD4^+$ T lymphocytes, increase in viral load, and poor disease prognosis. The neutralization of group O strains by M antibodies is as low as 34%, while neutralization of M by O antibodies remains inconsistent[64,65]. Comparisons of group O versus M antigenic targets by monoclonal neutralization antibodies have produced discrepant results, which could be related to the assay type. Early reports suggested that the monoclonal antibodies b12, 2F5, and 4E10 neutralized a few group O isolates[66]. However, a recent study of 12 group O isolates found all isolates were resistant to neutralization by these antibodies[65]. Further analyses with the highly efficient human monoclonal neutralization antibodies VRC01, VRC03,HJ16 PG9 and PG16 also demonstrated their inability to neutralize group O, with the exception of the latter two which neutralized two isolates[65].

The closest follow-up of disease progression in group O patients involved the $ANT70_A$ and $ANT70_B$ couple, who were closely monitored pre-antiretroviral therapy (pre-ART) for natural disease course until 1995. While the virus from $ANT70_A$ (virus donor) was later found to be X4 tropic, $ANT70_B$ (virus recipient) remained R5 tropic[67]. Similarly, in the Spanish group O couple $HIV\text{-}1_{ESP1}$ and $HIV\text{-}1_{ESP2}$, one of these patients remained asymptomatic for several years showing stable $CD4^+$ T-cells and NSI/R5 variants, while the partner progressed to AIDS with a corresponding switch from NSI/R5 to SI/X4[68]. Despite the description of these group O X4 tropic strains, phenotypic coreceptor and tropism studies of more than 30 group O isolates indicate an overwhelming dominance of R5 tropism, even during the late stages of infection, as reported for subtype M-C[69-72]. In fact, a case of a possible long-term non-progressor involving one of two group O patients living in the USA (JHG01-clade O-B) was reported recently[73]. Within a decade, this ART-naive subject's viral load remained undetectable (< 50 copies/ml), but the CD4 counts declined from 576 to 350 cells/ul[73].

HIV-1 tropism is influenced by the amino acid charge of the gp120 V3 loop. Group O V3 loops are longer than those of group M (average, 37 vs. 35 amino acids) irrespective of tropism (Table 2)[72]. Unlike group M where specific V3 loop amino acid residues are predictive of viral tropism, it is still unclear which residues determine tropism in group O. Current coreceptor prediction algorithms, such as Position Specific Scoring Matrix (PSSM), Geno2pheno (G2P), and WetCat, which were generated based on either group M-B or M-C sequences, are not sensitive enough to predict group O coreceptor tropism (Table 2)[72,74]. The G2P algorithm only correctly predicts about 50% of group O tropism, while PSSM wrongly predicts all group O strains as X4 (Table 2)[72]. In the WetCat and PSSM algorithms, some group O sequences cannot be recognized as HIV-1 V3 loop sequences due to their high genetic variability. Although the novel PhenoSeq algorithm has not been tested against group Os, all available *in silicio* algorithms are group M-based, non-sensitive to group O, generating results contrary to X4 and R5 phenotyping assays[75]. It is highly recommended that group O-specific algorithms be developed to facilitate reliable tropism prediction, enabling the use of entry inhibitors such as maraviroc for group O treatment.

HIV-1 group O and drug resistance

The main impact of HIV-1 group O sequence diversity on drug susceptibility involves its intrinsic resistance to some NNRTIs, caused mainly by the presence of a cysteine residue at position 181 of RT (C181)[47,76]. K103R

Table 2. HIV-1 group O and M V3 loop coreceptor tropism comparison using group M-based programs versus actual biological phenotype

Virus	(group: Clade)	V3 Loop sequence	V3 length	Charge total/net	Bio Pheno	11/25	PSSM*	geno2pheno† (FPR %)	MVC
		$11 $25							
CMO2.41	(O:A)	C E R P W N . Q T V Q **E** I R . I G . P M A W Y S M G I I . R E **E** K S S **N** L S R L A Y C	39	5/0	R5	R5	X4	??	+
YBF37	(O:A)	– – – – Y – . – – – I – – **–** V W . – – S – – – – – – – – A L D R E Q **N** – T I . . T – M – F –	39	1/0	R5	R5	X4	??	+
YBF39	(O:A)	– V – T G **–** . – – – – **Q** M K . V – . – – S – – – – A – S . H . **Q** K **N** D . . T – K – – –	36	5/4	R5	R5	X4	X4(1.7)	+
BCF07	(O:A)	– H – – G – . L K – – – **–** – K . – – . – – – – – – – – – – E A E N **I** P D . . . – – K – – –	37	6/2	X4/R5	R5	X4	X4(9.3)	+/–
BCF06	(O:B)	– M – K G R . G K I – **R** – A . T – . – L R – V – – A A K T E S **Q** N T G . . – – – I – – –	38	8/6	X4/R5	R5	X4	??	+/–
MVP5180	(O:B)	– I – E G I . A E – – **D** – Y . T – P . – R – R – – T L K . – S **N** N T – P R – – V – – –	39	6/4	X4/R5	R5	X4	X4(0.7)	+/–
		$11 $25							
Con B	(M)	C T R P N **N** N T R K **S** I H I G P G R A F Y T T G **E** I I G D I R Q A H C	35	7/5	R5	R5	R5	R5(24.7)	
RBF208	(O/M)	– V – – G **–** – – – R **–** – – F – – – – – – – – A N . **D** – T – – – – K – – –	35	8/6	R5	R5	R5	R5(23.6)	+
YBF30	(N)	– – – – G – – – G G **Q** V Q – – – A M T – – N I E **K** – V – – – – – – Y –	35	3/1	R5	X4	R5	R5(17.8)	+

*All group O sequences were depicted as X4 most likely because the program could not recognize the V3 loop sequence as real. †All group O sequences were accurately recognized as group O but were mostly predicted as X4.
FPR: false positive rate; *??*: program could not align sequences, indicates questionable results; All results from WetCat were non predictive and were left out of the Table; MVC: maraviroc. +/- IC_{50} of dual tropic viruses were the lowest. BioPheno: Biological phenotype/tropism on U87-CD4 CCR5 or CXR4 cells. Potential N-linked glycosylation sites are shaded (*modified from Tebit, et al. submitted*[72]).

is also found in some group O and is typically more common among the Y181 lineage (clade B, Fig. 3)[47,77]. This C181 amino acid located in the RT binding pocket causes resistance to NNRTIs, specifically nevirapine and etravirine, but has no effect on the RT activity and fitness[47,72]. No data is available on group O susceptibility to the novel NNRTI rilpivirine. Despite a 33% amino acid divergence between groups M and O RTs, the *in vitro* polymerase or RNase H kinetics of both groups are similar when the RNA and DNA templates are analyzed[78]. However, the RT of HIV-1 M-B seems to have a lower thermostability and a 2.5-fold lower efficiency in comparison to group O[79]. Further analyses of the evolutionary history of groups M and O RT has identified alterations in the solvent non-catalytic pocket of RT (p66 and p51 subunits), also known as the NNRTI binding pocket, as another distinguishable structural feature between both groups[47]. This pocket is also home to the NNRTI drug resistance mutations (Y181C, A98G, K103N and V179E), which segregate the group O lineages, as well as group O from M and other lentiviruses (Fig. 3)[47].

Susceptibility studies with novel drugs such as the integrase strand transfer inhibitors (INSTI), raltegravir, elvitegravir, and dolutegravir indicate that group Os are variably sensitive to these drugs, with a resistance pattern similar to group M[49,80]. Several group O-specific polymorphisms such as L74I, S153A, G163Q and T206S have been identified, but their impact on integrase inhibitor susceptibility in the context of a group O backbone is still unclear[72]. Both L74I and S153A are very close to the integrase catalytic site and may therefore affect the structure and efficiency of group O integrase[49]. Despite these acknowledged differences, INSTIs could be a valuable component in the first-line ART for group O patients, about 60% of whom are naturally resistant to NNRTIs, a major component of first-line treatment in Africa. Raltegravir has been effectively used as salvage therapy in group O patients failing various RT and PR regimens[81,82].

The current algorithms used to predict HIV resistance to ART are very robust, especially for group M-B and M-C strains. However, they are not suited for predicting resistance in group O, especially at drug resistance associated amino acid residues, which occur as natural polymorphisms[77]. Even among the three commonly used drug resistance algorithms developed by the ANRS, REGA, and the HIV Stanford databases, discrepancies in interpreting group O drug resistance exists[77]. Despite its diversity, detailed susceptibility studies of group O to novel protease inhibitors are still lacking and are necessary in order to validate the reliability of these group M-based drug resistance predicting algorithms[83].

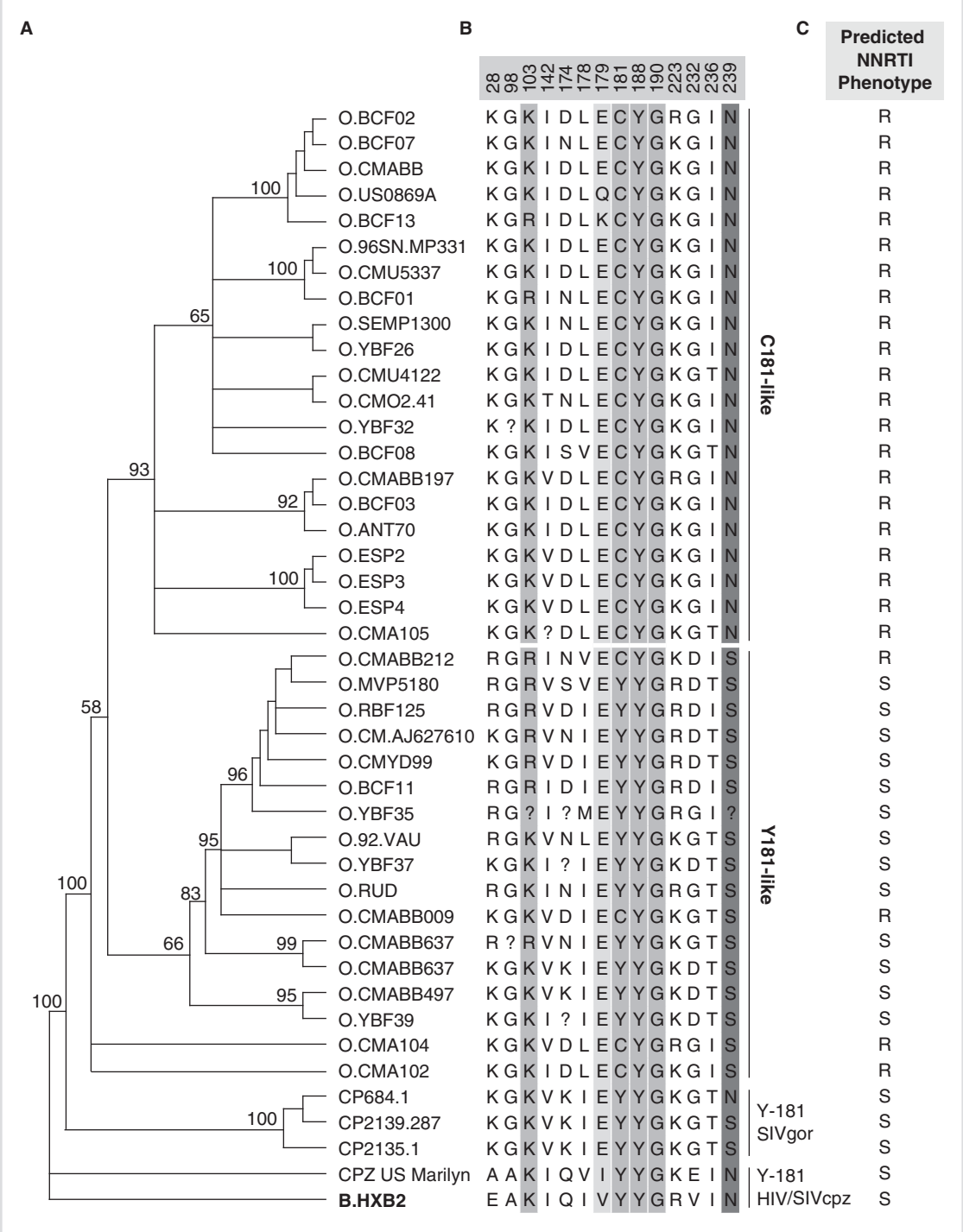

Figure 3. *Phylogenetic analyses of HIV-1 group O evolution in the reverse transcriptase gene.* ***A:*** *Neighbor joining phylogenetic analysis performed using the first 750 nucleotides from 38 HIV-1 group O and 3 SIVgor sequences.* ***B:*** *Signature amino acid residues were identified using VESPA and are grouped as C181-like (Clade A) or Y181-like (Clade B) sequences. The majority of group O sequences are C181-like, and the minority bears Y181 and cluster more closely with HIV-1 group M (HxB2). The amino acid positions are indicated at the top of the sequence. Question marks represent positions with no clear amino acid, as outlined in the Los Alamos database. Amino acids known to confer high- and low-level resistance to NNRTIs are indicated in light grey and grey, respectively. Completely conserved positions are shown in dark grey.* ***C:*** *Predicted NNRTI phenotypes are indicated as R (resistant) or S (susceptible). Figure was published earlier[47].*
NNRTI: nonnucleoside reverse transcriptase inhibitor.

The extreme sequence divergence of some group O drug targets might force these viruses to follow different genotypic resistance pathways as shown for HIV-2 and HIV-1 subtype C[84]. The low therapeutic success (59%) of group O patients under ART suggests that sequence diversity clearly influences the emergence of drug resistance[10]. Broad baseline susceptibility of group O to T20, a fusion inhibitor designed based on the heptad repeat 2 (HR2) region of the HIV-1 subtype B_{LAI} envelope gp41 sequence has been described[81,85]. Interestingly, the N42D mutation on the heptad repeat 1 (HR1) region associated with T20 resistance in group M occurs as wild-type sequence in 98% of group O isolates. N42D does not seem to affect group O susceptibility to T20 *in vivo* as it was shown to be active in a group O-infected patient carrying this mutation[77,86]. However, two residues 27A and 119E located in HR1 and HR2, respectively, are associated with major variation of the IC_{50} of group O strains[77]. These two mutations might influence the stability of the helix in gp41 or restore fitness by acting as accessory mutations. Similarly, a wide baseline susceptibility to maraviroc has been observed among 17 group O isolates[72]. The reason for and impact of such variation is not clear, but might be related to the entry and fitness of group O.

Restriction factor tetherin and group O fitness and spread

Tetherin (BST-2) is an interferon-inducible type II membrane glycoprotein that interferes with the late stage of HIV replication by preventing the release of nascent retroviral particles through physical tethering of their envelopes to the cell surface. Various SIV/HIVs use Vpu, Nef, or Env to counteract the activity of tetherin in a species-specific manner, indicating a selective evolution of these viruses (Fig. 1)[87,88]. While the Vpu of HIV-1 group M and some group N can counteract human tetherin[87], the Env of HIV-2 provides the anti-tetherin activity in its host (Fig. 1)[87]. Earlier reports suggested that the Vpu of groups O and P do not show any anti-tetherin activity[89]. This lack of activity was attributed to the inability of the group O Vpu transmembrane domain to bind or interact with tetherin as a result of its retention in the endoplasmic reticulum (ER) compared to group M Vpu which localizes to the trans-Golgi network (TGN). A single glutamic acid to lysine change at position 32 (E32K) of Vpu shifted the localization of group O Vpu from the ER to the TGN. However, this correction does not cause group O Vpu to antagonize tetherin[51]. Position 32 of Vpu is highly conserved, with about 95% of group O Vpu carrying a glutamic acid while lysine and valine both comprise only 5%[51].

The Nef protein of group O down-modulates human tetherin from the cell surface, but only modestly affect virus release (Fig. 1) [87,89,90]. The MRCA of group O Nef binds to a region immediately adjacent to a 5 amino acid deletion in the human tetherin, thereby down-modulating its expression by about 70% in both 293T-cells and human peripheral blood mononuclear cells (PBMC)[90]. This down-modulation increases virus resistance to interferon-alpha[90] and might have facilitated the spread of group O viruses in the human population, which relative to group M is limited geographically to West and Central Africa.

The confinement of group O to West and Central Africa in the HIV pandemic has been attributed in part to its low replicative fitness[91]. Several *ex vivo* fitness studies performed on human PBMCs between group O, HIV-1-M, and HIV-2 strains suggest that group O is the least fit among all HIV types and groups[91]. The difference in fitness ranges from 10- to 100-fold, depending on the group O strain analyzed[91]. In *ex vivo* infectivity assays on human tonsils, group M viruses replicated only about 5-8 times better than group O[92]. Between the two group O clades, there is no difference in fitness on PBMCs despite obvious differences in their susceptibility to some NNRTIs and CCR5 antagonist[72]. Due to the role of the HIV envelope in entry and fitness, it is possible that the diversity of group O and M envelopes might be a factor influencing the low fitness of group O.

Relevance of group O to the HIV epidemic and conclusion

The oldest identified cases of HIV-1 groups O and M date back to 1963 and 1959, respectively (Fig. 1)[19,38]. However, it remains unclear what role this divergence and significant sequence diversity of group O plays in its biology and pathogenesis. Limited follow-up studies of group O patients suggest a similar disease course as reported for group M[67]. It is however difficult to draw any conclusions by considering results from the limited and anecdotal follow-up studies that have been reported to date[67,68]. The dominance of R5 tropic strains in group O patients who were mostly at later stages of disease suggest that the coreceptor switch from R5 to X4 might be uncommon within this group. This would imply that group O patients progress slower to disease than M patients. With supporting data that (i) group O strains are dominantly R5 tropic, (ii) could be long-term-non progressors, and (iii) are the least fit among

all HIV types and groups, it is tempting to speculate that group O viruses might have evolved to levels less pathogenic than group M. Does this low fitness and pathogenesis suggest differences or defects in their accessory genes or structural genes such as env, gag, or pol? Clearly, more long-term prospective studies of group O subjects are required to provide more insights into the natural course of group O infection. Although studies involving group O pathogenesis will be difficult to perform as current protocols recommend the administration of ART at a relatively early disease stage prior to the onset of AIDS, it is still possible to piece together current available *in vivo, in silicio,* and *ex vivo* data to better understand group O pathogenesis.

Clearly, group O viruses have greatly enlightened us about HIV evolution and interspecies transmission and will continue to do so. Reports of dual O/M infections and recombinants are of concern as patients receiving treatment might be reactive for group M envelope antibodies but carry group O protease or RT that are less susceptible or resistant to current antiretrovirals. Continuous monitoring of group O and related strains is therefore important for the design of any future therapeutic and/or vaccine strategy. Group O strains occurred relatively early in the HIV pandemic alongside group M, suggesting that just like group M, this group had an equal opportunity to spread. Its limited spread could be due to viral and/or host factors. Understanding how viral factors influence pathogenesis in group O will require extensive *ex vivo* phenotypic studies associated with immunological and virological follow-up of a group O patient cohort. Results from such studies will guide our efforts to curb the spread of the more pandemic HIV-1 group M and novel variants that might emerge in future. Until such a time, the burning question of why group O is limited mostly to Central Africa, despite its ability to be transmitted both heterosexually and vertically, remains.

Declaration of Interest

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