AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 14, Number 11, 1998, pp. 951–961
Mary Ann Liebert, Inc.

# Genetic Characterization of Accessory Genes from Human Immunodeficiency Virus Type 1 Group O Strains

FRÉDÉRIC BIBOLLET-RUCHE,[1] IBTISSAM LOUSSERT-AJAKA,[2] FRANÇOIS SIMON,[2] SOULEYMANE MBOUP,[3] EITEL MPOUDI NGOLE,[4] ERIC SAMAN,[5] ERIC DELAPORTE,[1] and MARTINE PEETERS[1]

## ABSTRACT

**Human immunodeficiency virus type 1 (HIV-1) group O strains have been described as highly divergent, compared with the vast majority of the viruses involved in the worldwide AIDS pandemic, classified in group M. To gain new insights into the diversity and genetic characteristics of group O, we have sequenced the accessory gene region (from *vif* to *vpu*) of 14 isolates. Analyses of the deduced amino acid sequences for Vif, Vpr, the first exon of Tat, and Vpu indicate that most of the functional domains of these proteins, as described for group M viruses, are highly conserved and retained among all the group O strains we have characterized. The only difference concerns the Vif phosphorylation sites, which are absent in all of the group O isolates we have sequenced; in contrast, they are well conserved in nearly all of the group M isolates, in which they play critical roles in the regulation of viral replication and infectivity. As already observed for group M isolates, the Vpu protein is also highly diverse among group O strains. Phylogenetic analyses of these sequences indicate that HIV-1 group O can be separated into four different clusters, containing most of the strains we have characterized (except one, which clusters outside of the analyzed viruses). Taking into account the criteria used for clades in group M, we were not able to define group O clades definitively.**

## INTRODUCTION

In 1990, an unusual human immunodeficiency virus type 1 (HIV-1) strain, isolated from West–Central African patients, was partially sequenced[1] and found to be highly divergent from previously described HIV isolates. Complete sequencing of the genome of this virus (ANT-70), and of a virus isolated more recently (MVP5180), revealed that they share the same genomic structure as previously characterized HIV-1 strains, as indicated notably by the presence of the *vpu* gene.[2,3] HIV-1 viruses were then divided into two groups: group M (for major), comprising the vast majority of strains responsible for the worldwide AIDS pandemic, and group O (for outlier), containing highly divergent HIV-1 strains, mainly found in patients of West–Central African origin (Cameroon and Gabon).[4,5] Interestingly, these two HIV-1 groups were approximately equidistantly related to the nonhuman primate counterparts of HIV-1 found in chimpanzees, SIVcpz-gab[6] and SIVcpz-ant.[7] More recently, HIV-1 group M has been further divided into at least 10 distinct clades, designated subtypes or genotypes A through J.[8,9] Each is approximately equidistantly related in phylogenetic analyses and is thought to have diverged from a common ancestor. These different subtypes are not equally distributed geographically: all of them were found in Central Africa with different prevalence, the A subtype being predominant in most African countries, whereas the B subtype was initially found mainly in developed countries (North America and Europe; for a review see Ref. 10). Several epidemiological studies have shown that all of the subtypes have now started to spread throughout the world; for example, non-B subtypes have emerged in developed countries, representing an important proportion of the new cases of heterosexual infection.[11–13] Although no clear correlation between HIV-1 group M subtypes and their pathogenicity has been established, some controversial results indicate that some

[1]Projet SIDAK, Laboratoire Rétrovirus, ORSTOM, 34032 Montpellier cedex 1, France.
[2]Laboratoire de Virologie, CHU Bichât Claude Bernard, 75018 Paris, France.
[3]Projet SIDAK, Hôpital A. Le Dantec, Université Cheik Anta Diop, Dakar, Senegal.
[4]Centre Hospitalier Militaire, Yaoundé, Cameroon.
[5]Innogenetics, B9052 Ghent, Belgium.

differences could exist between the subtypes concerning their transmissibility.[14–16]

The prevalence of HIV-1 group O is low compared with that of group M, accounting for 3.3% of the HIV-1 infections in Cameroon,[5] which is considered to be the country with the highest prevalence in Africa.[17] However, even if West–Central Africa seems to be the main reservoir for these highly divergent viruses, they have been detected in several other parts of the world, including West and East African countries,[17,18] Europe,[19–22] and the United States.[23] Genetic characterization of HIV-1 group O strains, mainly in the envelope (C2V3 region) and the *gag* genes, indicates that they are genetically diverse and no phylogenetic clustering pattern similar to that of HIV-1 group M subtypes has been found.[21,24–26] This result could be due to an artifact of sample size, since only a few sequence data are available compared with group M, or could be because the length and/or regions of the group O genomes that have been studied are not informative enough to reveal phylogenetic associations within these viruses.[26]

Here, we report the genetic characterization of the accessory genes region (from *vif* to *vpu*, approximately 1330 bp) for 14 HIV-1 group O strains. These genes were considered as nonessential for the pathogenicity of HIV, but their high conservation and data concerning their role during pathogenesis *in vitro* and *in vivo* suggest that their role has probably been underestimated.[27–36] Moreover, several results have shown that they could constitute interesting targets for antiretroviral therapy.[37–41]

## MATERIALS AND METHODS

### *Viruses*

Most of the strains used in this study have been described previously: BCF01, -02, -03, -06, -07, -08, and -11,[21] and BCF09, -12, and -13,[42] have been isolated from Cameroonian patients living in France. the GAVI686 isolate is from a Gabonese patient[43] and 95SNMP331 is from a Senegalese patient infected through sexual contact with her partner (who was probably infected in Cameroon).[18] New isolates include the 96CAMP539 strain, isolated from a Cameroonian patient by coculture of his peripheral blood mononuclear cells (PBMCs) with seronegative donor PBMCs as described previously,[13] and 95CAMP448, which was amplified directly from uncultured PBMCs of a Cameroonian patient. To the best of our knowledge, none of the patients from whom these viruses have been isolated are epidemiologically linked.

### *Polymerase chain reaction amplification and sequencing*

DNA from cultured and uncultured PBMCs was extracted using IsoQuick (Microprobe, Garden Cove, CA) according to the manufacturer instructions and quantified spectrophotometrically. Approximately 1 $\mu$g of DNA was used for a first round of amplification with an outer primer pair (VIF1, 5′ GGGTTTATTACAGGGACAGCAGAG 3′; VPU1, 5′ GGTTGGGGTCTGTGGGTACACAGG 3′) in a final volume of 100 $\mu$l of containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, a 0.2 mM concentration of each deoxynucleoside triphosphate, 2.5 U of *Taq* DNA polymerase (Promega, Madison, WI), and a 0.4 $\mu$M concentration of each primer. Five microliters from this first round was used for a second round with an inner primer pair (VIF2, 5′ GCAAAACTACTCTGGAAAGGTG 3′; VPU2, 5′ GCWTCTTTCCACACAGGTACCCC 3′; W = A or T) under the same reaction conditions. The two rounds were run under the following cycling conditions: 94°C for 30 sec, 50°C for 30 sec, and 72°C for 2 min, for 35 cycles, preceded by a denaturation step of 3 min at 94°C and followed by a final extension step of 7 min at 72°C. Sequencing of the amplified products was done directly after purification by tris acetate EDTA (TAE)–low melting point agarose gel electrophoresis, as described previously,[44] using an Applied Biosystems (Foster City, CA) 373 Stretch sequencer and a Dye-Deoxy terminator procedure (dye terminator cycle sequencing ready reaction, with AmpliTaq DNA polymerase; Perkin-Elmer, Norwalk, CT) as specified by the manufacturer. Inner polymerase chain reaction (PCR) primers (VIF2 and VPU2) and inner sequencing primers (OVIF, 5′ CATATTGGGGATTGATGCCAG 3′; OVPU, 5′ GCATYAGCGTTACTTACTGC 3′; Y = C or T) were used. Overlapping sequences were joined using SeqEd (Applied Biosystems) to obtain the full-length sequence. Because the main goal of this study was to obtain new insights into HIV-1 group O genetic diversity, only direct sequencing was performed. Ambiguities observed at a limited number of positions in some sequences were resolved when joining the overlapping fragments.

### *Analyses of accessory protein sequences*

Open reading frames for the different accessory proteins (Vif, Vpr, the first exon of Tat, and Vpu) were determined and the deduced protein sequences were aligned using CLUSTAL W.[45] Consensus sequences were determined for each of them and were manually aligned to the available corresponding consensus sequences fro HIV-1 group M.[9] Secondary structure predictions were determined according to programs developed by J.-F. Gibrat, J. Garnier, and B. Robson.[46,47]

### *Phylogenetic analyses*

DNA sequences were aligned by using CLUSTAL W[45] with minor manual adjustments, bearing in mind the position of the open reading frames and the predicted amino acid sequences. Regions with a gap in any of the sequences were excluded from the analyses. Phylogenetic relationships between the HIV-1 group O strains were determined by the neighbor-joining method[48] with the Kimura two-parameter distance matrix.[49] Reliability of the branching order was estimated from 1000 bootstrap replicates.[50] These methods were implemented by using CLUSTAL W.[45] Tree topologies were also inferred by the maximum parsimony method with repeated randomized input order, implemented using the DNAPARS program from the PHYLIP package.[51] The results obtained by both methods were the same in all essential aspects. Unrooted phylogenetic trees for group O viruses were constructed by the neighbor-joining method using SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE, and viewed using DRAWTREE from the PHYLIP package.[51]

Deduced protein sequences for Vif, Vpr, Tat, and Vpu were concatenated for each sequenced strain and aligned using

CLUSTAL W. Phylogenetic relationships were determined using SEQBOOT and PROTDIST, followed by NEIGHBOR or PROTPARS (with repeated randomized input order), and CONSENSE from the PHYLIP package.[51]

### *Nucleotide sequence accession numbers*

The sequence are available through EMBL. The accession numbers are as follows: Y16030, BCF01; Y16021, BCF02; Y16022, BCF03; Y16026, BCF06; Y16029, BCF07; Y16024, BCF08; Y16023, BCF09; Y16018, BCF11; Y16031, BCF12; Y16020, BCF13; Y16025, 95SNMP331; Y16028, 95CAMP448; Y16007, 96CAMP539; Y16019, GAVI686.

## RESULTS

### *Nucleic acid and protein sequences*

We have successfully amplified with high efficiency (allowing direct sequencing, as described in Ref. 44) all of the HIV-1 group O viruses we have tested, either from isolated strains or from uncultured PBMCs, using outer and inner primers we have designed. The length of the sequenced fragments varied from 1327 bp (BCF03) to 1342 bp (BCF02, BCF07, and 95CAMP448) owing to insertion of 3 nucleotides in *vpu* or to deletions in *vpr* and *tat* genes (see Fig. 4). Comparisons of the sequences indicates that this accessory genes region is well conserved among HIV-1 group O strains, with divergence ranging from 4.6 to 16.4% (mean, 11%), values that are comparable to the divergence observed between HIV-1 group M isolates (6.6–18.4% [mean, 12.5%]; see Table 1, footnote a, for group M viruses used for calculation). In this part of the viral genome both HIV-1 O and M groups are more closely related to SIVcpz-gab (mean divergence, 30.2 and 27.5%, respectively) than to SIVcpz-ant (mean divergence, 36.5 and 36.4%, respectively). Genetic divergence was also calculated individually for the different accessory genes, *vif, vpr, tat,* and *vpu* (Table 1). For both HIV-1 groups, *vpu* is the most divergent gene (mean divergence, 16 and 20.8% within group O and M, respectively) and the mean divergence for the other genes (*vif, vpr,* and *tat*) is comparable (approximately 9 to 13% for group O and M, respectively). Comparison of the two HIV-1 groups with the viruses from chimpanzees[6,7] confirms this observation and also shows that *vpr* and *tat* genes from groups O and M are more homologous to SIVcpz-gab than to SIVcpz-ant. On the other hand, group O and M *vpu* genes are approximately equidistant from SIVcpz-gab and SIVcpz-ant. Comparison of the *vif* genes indicates that in group M it is more similar to SIVcpz-gab (mean, 25.5%) than to SIVcpz-ant (mean, 33.8%) while in group O, *vif* is equidistant from both chimpanzee viruses (mean, 31.4 and 34.8% to SIVcpz-gab and SIVcpz-ant, respectively).

Genetic divergence was also calculated for individual protein sequences (Table 1). As for nucleic acid sequences, intragroup divergence for group O accessory proteins is lower than for group M, and both groups are more closely related to SIVcpz-gab than to SIVcpz-ant. Vpu protein divergence cannot be considered among the different lineages since it was not possible to align these proteins correctly, except in the domain containing the phosphorylation sites (where the SIVcpz-gab se-

TABLE 1. PERCENT GENETIC DIVERGENCE[a]

| | *Nucleic acid sequences* | | | *Amino acid sequences* | | |
|---|---|---|---|---|---|---|
| *Virus* | *Genes* | *Group O* | *Group M* | *Proteins* | *Group O* | *Group M* |
| Group M | *vif* | **28.5** | **12.3** (6.0–14.5) | Vif | **36.1** | **18.2** (6.8–24.1) |
| | *vpr* | **25.1** | **13.0** (5.2–18.2) | Vpr | **24.9** | **17.6** (4.2–26.8) |
| | *tat* | **27.0** | **14.2** (8.2–18.3) | Tat | **37.4** | **22.4** (7.3–29.2) |
| | *vpu* | **38.5** | **20.8** (12.5–25.8) | Vpu | **>70** | **31.8** (12.3–39.0) |
| Group O | *vif* | **10.1** (4.1–15.5) | | Vif | **15.6** (6.8–23.4) | |
| | *vpr* | **8.2** (3.0–13.2) | | Vpr | **11.4** (3.0–17.0) | |
| | *tat* | **9.6** (2.8–15.7) | | Tat | **14.1** (2.8–22.6) | |
| | *vpu* | **16.0** (6.2–23.9) | | Vpu | **26.3** (7.8–44.0) | |
| SIVcpz-gab | *vif* | **31.4** | **25.5** | Vif | **38.9** | **33.6** |
| | *vpr* | **23.4** | **23.7** | Vpr | **27.9** | **22.5** |
| | *tat* | **25.1** | **22.2** | Tat | **34.6** | **34.2** |
| | *vpu* | **38.0** | **36.4** | Vpu | **>70** | **>70** |
| SIVcpz-ant | *vif* | **34.8** | **33.8** | Vif | **49.9** | **44.3** |
| | *vpr* | **33.0** | **34.6** | Vpr | **N/A** | **N/A** |
| | *tat* | **30.7** | **32.3** | Tat | **38.6** | **42.8** |
| | *vpu* | **36.1** | **39.2** | Vpu | **>75** | **>70** |

[a]Intra- and intergroup divergence for nucleic acid and protein sequences (determined by pairwise alignment and comparison) for the different accessory genes and proteins Vif, Vpr, first exon of Tat, and Vpu, also compared with the corresponding genes for the two chimpanzee viruses SIVcpz-gab and SIVcpz-ant. Group M sequences used for calculation include nonrecombinant A (U455, 92UG037), B (LAI, WEAU, HAN, MANC), C (C2220, 92BR025), D (ELI, NDK, Z2Z6), F (93BR020), and H (90CF056) subtype sequences available from the Los Alamos HIV database.[9] Mean values are indicated in boldface and minimal and maximal values are indicated in parentheses.

*Abbreviation*: N/A, SIVcpz-ant Vpr is truncated by in-frame stop codons, and therefore was not included in comparisons.

quence is identical to group M [EDSGNES] and different from group O [DDSYES]), leading to divergence values higher than 70%. Similar to nucleic acid sequence results, group M, Vif, Vpr, and Tat sequences are more homologous to SIVcpz-gab than to group O or SIVcpz-ant (Table 1).

### *Phylogenetic analyses*

Phylogenetic trees were constructed from the 14 new sequences described here and the two fully characterized HIV-1 group O strains ANT70[3] and MPV5180,[2] using SIVcpz-ant as an outgroup. We have also included sequences available for group M clades including nonrecombinant A, B, C, D, F, and H subtypes,[9] as well as SIVcpz-gab. Neighbor-joining and maximum parsimony methods were used, as described in Materials and Methods, to construct trees from nucleotide sequences; both methods give consistent results and the neighbor-joining tree is shown in Fig. 1. It is apparent that the different strains can be split into at least four clusters (designated a, b, c, and d in Fig. 1), supported by bootstrap values of more than 80%. To test the robustness of these different clusters, unrooted phylogenetic trees were constructed for group O sequences from contiguous overlapping (100-bp) segments of 400 bp. Results are shown in Fig. 2 and indicate that a and d clusters are reproducibly found along the whole accessory gene region, supported by bootstrap values of more than 75%. The two other clusters are not always well separated in the *vif-vpr-tat* region, mostly owing to uncertain branching orders of BCF09 and GAVI686 in these genes. Rather than suggesting that these strains have a recombinant genome as observed for group M subtypes,[9] these discrepant branching orders probably reflect independent evolution since these strains turn to nonclassified strains instead of grouping with the clusters. In each tree, the 96CAMP539 isolate is clearly distinct from the four clusters, suggesting that other clusters may exist. Phylogenetic trees constructed from the alignment of the concatenated protein sequences for Vif, Vpr, Tat, and Vpu yield identical patterns for the branching orders (data not shown). On the other hand, group M clades are clearly discriminated by phylogenetic analysis of this accessory gene region (Fig. 1), and consistent with genetic divergence data, SIVcpz-gab clusters close to group M isolates.

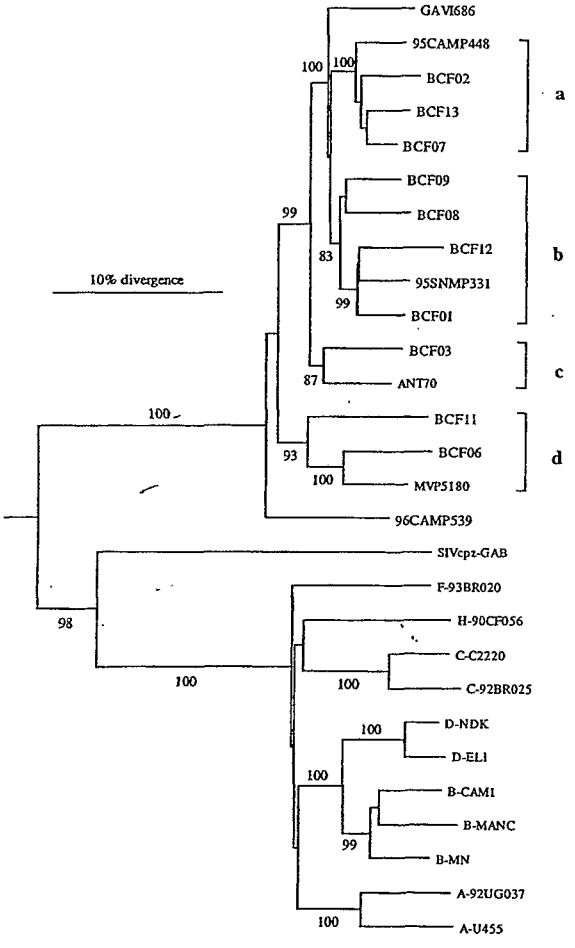


**FIG. 1.** Phylogenetic relationships of full-length accessory gene sequences of the newly characterized HIV-1 group O strains (indicated in boldface), by the neighbor-joining method. This tree was rooted using SIVcpz-ant as an outgroup. Horizontal branch lengths are drawn to scale (the scale bar represents 0.1 nucleotide substitution per site), while vertical separation is for clarity only. Values at nodes indicate the percentage of bootstraps (1000 resamplings) in which the cluster to the right was found (values of 80% and higher are shown). Brackets at the right indicate the four group O clades a, b, c, and d.

To explore further these phylogenetic relationships, genetic distances calculated according to the Kimura two-parameter method were compared (data not shown). Mean values for intra- and interclade distances for group M are 0.07 and 0.19, respectively (Table 2). Similarly, the intracluster mean distance for group O is within the same range as the group M intraclade distance (0.08), but in contrast the intercluster distance for clusters a, b, and c is also approximately 0.1. On the other hand, results suggest that strains grouped within these clusters (and GAVI686) can be gathered together into the same cluster, but on the other hand, a value of 0.11 is close to that of the group M B/D clade division in this accessory gene region (mean genetic distance of 0.12). Strain 96CAMP539 and cluster d are more distantly related to the a, b, and c clusters, with mean genetic distances of 0.16 and 0.18, values that are close to the group M interclade mean distance (0.19).

### *Comparison of deduced amino acid sequences*

An alignment of the deduced amino acid sequences for each of the different accessory proteins Vif, Vpr, the first exon of Tat, and Vpu is shown in Fig. 3. As already noted for nucleic acid sequences, Vpu is the most variable protein (mean divergence, 26.3%; ranging from 7.8 to 44%), with important diversity in the N-terminal part of the protein. In group M this region has been shown to form an $\alpha$-helix structure[52]; calculation of the folding of Vpu for group O strains indicates that this secondary structure is also found in all the isolates (data not shown), suggesting a conserved function despite the important variability. The other proteins (Vif, Vpr, and the first exon of Tat), are well conserved among group O isolates and no particular features can distinguish the strains belonging to the different clusters defined by the phylogenetic analyses. Among group O strains the mean divergence for Vif is 15.6% (6.8–23.4%), for Vpr 11.4% (3–17%), and for the first exon of Tat 14.1% (2.8–22.6%), and these values are similar to the mean

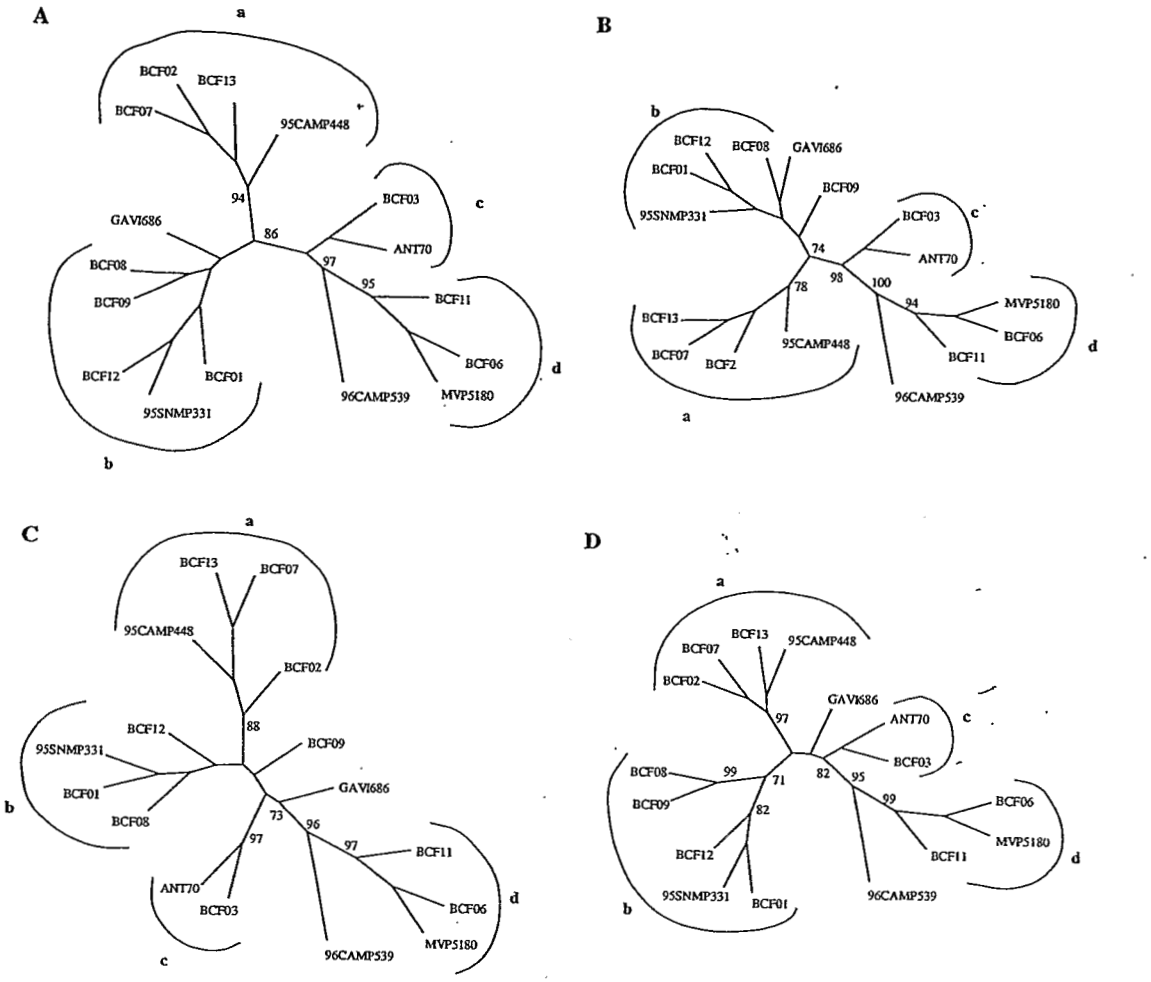


**FIG. 2.** Unrooted neighbor-joining trees constructed for a 400-bp fragment moved in increments of 100 bp along the group O sequence alignment. The four clusters a, b, c, and d (see Fig. 1) are identified. Branch lengths are arbitrary and do not scale. The numbers at the nodes indicate the percentage of bootstraps (100 resamplings) supporting the adjacent cluster (values of 70% and higher are shown). (A) 5′ *vif*; (B) 3′ *vif*-5′ *vpr*; (C) *vpr*-5′ *tat*; (D) *tat-vpu*.

divergence among group M isolates. The Vif protein contains 192 amino acids in all the strains analyzed, while a deletion of 3 amino acids (SNT at positions 86–88 in the consensus sequence) is found in the C-terminal part of Vpr in two isolates (ANT-70 and BCF03), and a deletion of one arginine residue at position 54 in the Tat protein is observed in strains ANT-70, BCF01, BCF12, and BCF03.

Consensus protein sequences were deduced for each accessory protein from the global alignment (consensus sequences for the group O clusters were almost identical) and were aligned to the corresponding consensus sequences available for group M genotypes A, B, and D.[9] Alignment of the consensus sequences is shown in Fig. 4, where critical residues or domains described to be important for the accessory protein functions in group M strains are also indicated. For most of these features, comparison of the consensus sequences indicates that group O accessory proteins could probably have the same functions as in group M, since secondary structures, cysteine residues, or domains with a particular composition are well conserved. The only important difference that can be observed concerns the

TABLE 2. KIMURA TWO-PARAMETER GENETIC DISTANCE[a]

| *Viral group* | *HIV-1 subtypes or clusters* | *Distance* |
|---|---|---|
| Group O | Intraclusters (a, b, c, d) | 0.08 |
| | a versus b | 0.10 |
| | a versus c | 0.11 |
| | b versus c | 0.11 |
| | a, b, c versus d | 0.16 |
| | a, b, c versus 96CAMP539 | 0.16 |
| | d versus 96CAMP539 | 0.18 |
| Group M | Intraclade | 0.07 |
| | B/D | 0.12 |
| | Interclade (others) | 0.19 |
| Group O/group M | | 0.48 |
| Group O/SIVcpz-gab | | 0.45 |
| Group M/SIVcpz-gab | | 0.44 |

[a]Mean genetic distance, calculated according to the Kimura two-parameter method, between all the accessory gene regions for group O isolates and for the HIV-1 group O clusters (a, b, c, and d), compared with group M intra- and interclade divergence. For comparison, group O/group M, group O/SIVcpz-gab, and group M/SIVcpz-gab mean distances are indicated.

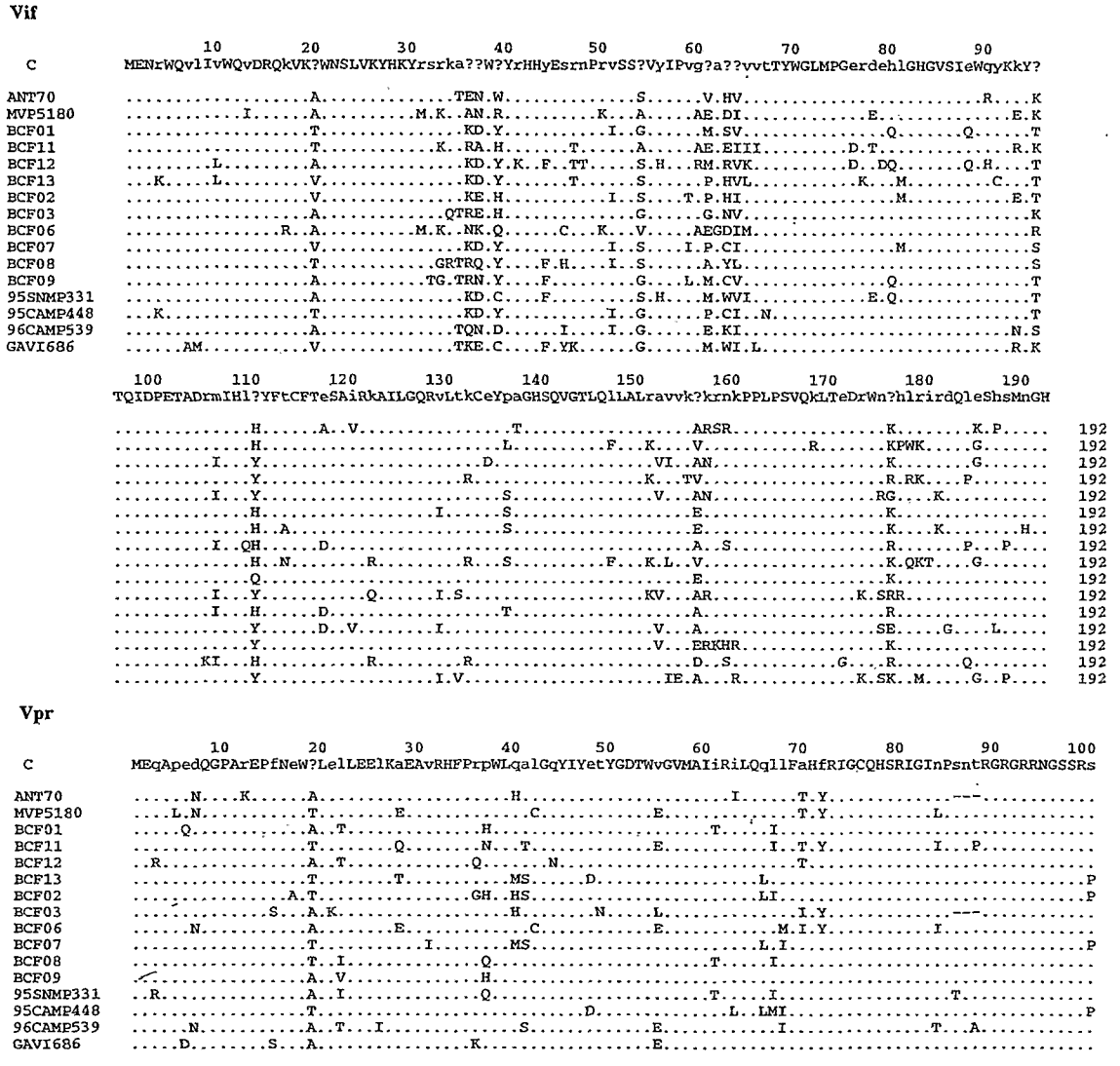


**FIG. 3.** Alignment of the deduced amino acid sequences for Vif, Vpr, the first exon of Tat, and Vpu accessory proteins of the group O strains characterized in this study, compared with available data for ANT-70 and MVP5180 group O isolates. Dots denote sequence identity with the consensus sequence (C, indicated at the top for each protein), while dashes represent gaps introduced to optimize alignments. Upper-case letters refer to amino acid residues that are conserved in 100% of the sequences, and lower-case letters represent amino acid residues covered in at least 50% of the sequences. Question marks in the consensus sequences indicate sites at which less than 50% of the viruses share the same amino acid residue.

phosphorylation sites in Vif. Three phosphorylation sites are found in group M isolates S144, T170, and T188), and their recognition motifs R/*KXXS*/T or R/*KXXXS*/T are used by serine/threonine protein kinases such as cGMP-dependent protein kinase and protein kinase C (PKC). In contrast, in group O isolates, such recognition sites are not found since lysine residues (K141 and K167) are replaced by glutamine residues (Q) in the two phosphorylation sites at positions 144 and 170, and by a leucine residue (L) instead of an arginine residue (R184) in the last site at position 188. These features suggest that if phosphorylation in group O strains is taking place, protein kinases other than those involved in group M viruses may be involved.

## DISCUSSION

Among the two HIV-1 groups, designated M and O, the latter remains poorly studied, essentially because of the limited availability of the isolates. Genetic characterization of the C2V3 region indicates that group M O viruses are more diverse compared with group.[5,21,23] To attain new insights into the genetic diversity and characteristics of HIV-1 group O we have sequenced the accessory gene region of 14 isolates.

Our data show that most of the structural or functional domains involved in the functions of accessory proteins described for group M strains are also likely to be present in group O

**Tat (first exon)**

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                10        20        30        40        50        60        70
 C      MDPVDPempPWHhPGSqPqtPCN?CyCK?CCYHCyvCFt?KGLGISyGRKKRrrpaaaas?pdnkD?vpeQ

ANT70   .......V..........I...N....R.........VR...........G-RP....H..H..PVPKQ   70
MVP5180 ..............K.....N....R.........K.....H.............Y.....P....   71
BCF01   ......................K.F..K.........R.............-RP...RH.....L....   70
BCF11   ......................Q....Y.....L....K..................GRHQ....PIS..   71
BCF12   ...................N...K....K.........R.............-RT...HH..H..LP...   70
BCF13   ............Q.....P....N....K.........S.....................R.....L....   71
BCF02   .......L..........PN...N....K.........S.....H.........T...R.....I....   71
BCF03   .......VS.............T....S.........R.............-RH....H.VHQ.P....   70
BCF06   .......I........K.....N....R.....F....K.....H.............Y.....P....   71
BCF07   ................PN...N....K.........S.....................R.....L....   71
BCF08   ......................K....K.....C....S.....................H.....L....   71
BCF09   ......K...............T....K.........S.....................H.....L....   71
95SNMP331 ...................I...N....R.....L....R.............QRR....H.....L....   71
95CAMP448 ...................N...K....K.........S.....................R.....L....   71
96CAMP539 .......V..........P....A....R.....L...K.....H.............SSN...L....   71
GAVI686 ......................N....Y.........S.....................G.RR.....PI...   71
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**Vpu**

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                10        20        30        40        50        60        70        80
 C      Mhh?dLl?liiisaLllin?i?W?f?Lr?yLeq?kQdrrereileRlrRirei?DDSDYeSn?eeeQEv??dLvhs?GFdNPmFEl

ANT70   ...R...AI.......F..V.L.G.I..K....KE...K.............R........G......M-...L.H........P   85
MVP5180 ..QEN..A..AL...C...VLI.L.N..I..V.R......Q.........K..R........E..Q...M-E.I..H..A.....   85
BCF01   ...K...IV..........V.I.M.N..Q...ER.....G...V.........K........E......R-..I..H.........   85
BCF11   ..QR...A..ACIF.C.L.A.F.L.N..L...RRR............I......G.....G.....IM-.....H........F   85
BCF12   ...S..-PTL.........V.L.M.I..QC.KNK.................TQ.....ED.....R-.....H.........   84
BCF13   ...K...L..V.L.....I.L.R.N..K...HKE.................R........E......R-.....TF.........   85
BCF02   .L.RN..LS.........T.I.L.MYI..R....R...S.............R........E......RGQ...NF.....     86
BCF03   .Q.K...IV..S....F..V.L.TYN.KT....R.............E...K.R........G......M-.....Y..A.....   85
BCF06   ..QR...AI.AL...C..SI.V.IIN..N....R......Q.........V...K.....D..G......M-..M..H........W   85
BCF07   ...R...L.V..........I.I.M.N.SK...PK.................R........GGGG...RGH...TF..A.....I   86
BCF08   .Q.R...T...........V.L.M.V..QC...K..N.....I.....L...K........G..G...R-.....Y.........   85
BCF09   .Q.R...T...T.......V.I.M.V..LC...RR........IR........E........G......R-.....H.........   85
95SNMP331 ...R...V...........V.I.M.I..Q....K.......D........A..K........E......R-..I..H.....     85
95CAMP448 .QQK...L.V..........I.L.M.N..K....K.............I.....R........E......RGH...MF..A..V..I   86
96CAMP539 ...K...I..VA.I..FT.IVI.T.I.KK....KE.......L.K.IK....VR.........GDGG...I-H...TH..V.....   85
GAVI686 ...R...T.........FL.V.L.T.V.NK....K.................K.......DG......M-....DY.........   85
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**FIG. 3.** *Continued.*

viruses. These domains include secondary structures such as $\alpha$-helix domains in the N-terminal region of Vpr and Vpu,[52,53] charged domains such as acidic and basic domains of Tat[54,55] and basic residues in the C-terminal end of Vpr,[56] specific motifs such as SL(V/I)$X_4$YX$_9$Y at the beginning of Vif[57] and H(S/F)RIG repeats,[58] the conserved GC peptide[59] and leucine zipper-like domain[60] in Vpr, critical residues such as cysteines in Vif and Tat,[61–63] and phosphorylation sites in Vpu.[64,65] The only difference that can be observed concerns the residues responsible for recognition of the phosphorylation sites in the Vif protein (R/K*XXS*/T* or R/K*XXXS*/T*), which are absent in all group O isolates we have characterized. In group M, phosphorylation of these sites (probably by PKC, which has already been shown to have several roles in HIV-1 replication[66–68]) has been shown to play a critical role in the regulation of replication and infectivity.[69] For example, removal of the first site (S144) results in a loss of Vif activity and more than 90% inhibition of viral replication.[69] Interestingly, these results can be directly linked to the observation that cyclophilin A (CypA) is required for the replication of group M strains and SIVcpz-gab but not for group O or other primate immunodeficiency viruses (including HIV-2, SIVmac from macaques, and SIVagm from African green monkeys[70]). Analysis of the Vif recognition phosphorylation sites of these different viruses indicates that the first site is absent in all of the viruses that do not require CypA for replication and is present in group M and in SIVcpz-gab.[9] CypA has been shown to be specifically incorporated into HIV-1 virions through binding to p24$^{gag}$[71,72] and to interact with other virally encoded proteins such as Nef, Vif, and gp120.[73] Interestingly, the binding affinity of Vif for CypA is similar to that of Vif for p24, indicating that this interaction is likely to occur *in vivo*.[73] Vif has also been shown to be incorporated into viral particles[74] and to colocalize with Gag at the plasma membrane of infected human T cells.[75] Whether a ternary complex between p24, CypA, and phosphorylated Vif is relevant *in vivo* has not been addressed, and further experiments are necessary to have a better idea about the functions of these different proteins in the regulation of viral protein assembly or viral budding. Nevertheless, data presented here indicate that, although the two HIV-1 lineages have evolved independently, some features are probably inherited from a common ancestor and hence are found in both clusters. The accessory proteins seem to be a well-conserved viral feature and could provide interesting targets for new antiretroviral therapies on the basis of the high conservation of the accessory proteins.

Comparison of *vif* genes from the two HIV-1 clusters with the chimpanzee viruses indicates that group M is more closely related to SIVcpz-gab (both presenting phosphorylation sites in Vif[9]) than to group O, while both O and M groups are equidistantly related to SIVcpz-ant (which does not have phosphorylation sites in Vif). Similarly, Vpr and Tat proteins from group O and M are more related to SIVcpz-gab than to SIVcpz-ant, and the Vpu protein is as divergent between group M, group O, SIVcpz-gab, and SIVcpz-ant. Concerning the hypothesis of a common ancestor for all these lineages, such observations suggest that these genes have evolved at different rates, which is conceivable since these different proteins play distinct roles

during HIV replication. Vpu is involved in two independent physiological activities: the first is its role in the release of virus particles, which could be achieved by its ability to form cation-selective ion channels[76] through its N-terminal part ($\alpha$-helix domain); the second involves the degradation of the CD4 molecule in the endoplasmic reticulum via a phosphorylation-dependent mechanism.[77] Other viral proteins could have the same activities, since Nef has been shown to downmodulate the expression of CD4 at the cell surface[77] and Vpr has also been shown to form cation-selection ion channels[78] with the same selectivity as Vpu channels. In fact, both known Vpu activities could be considered as redundant with other viral protein activities and could therefore be considered as the "most accessory" protein. To develop a more definitive view of the role of the Vpu proteins, it will be necessary to compare their function in the different virus lineages carrying this highly diverse protein.

Analysis of accessory gene sequences clearly discriminates group M and group O isolates. Genetic diversity within both groups is comparable, suggesting that these two lineages have evolved for equivalent periods of time. Accessory genes could be of particular interest in studying the evolution of lentiviral genes, since they are poor targets for natural antibodies (in fact, some antibodies have been detected but not in all patients) and therefore are subjected to limited pressure by antigenic selection. We cannot exclude the possibility that there may be cy-

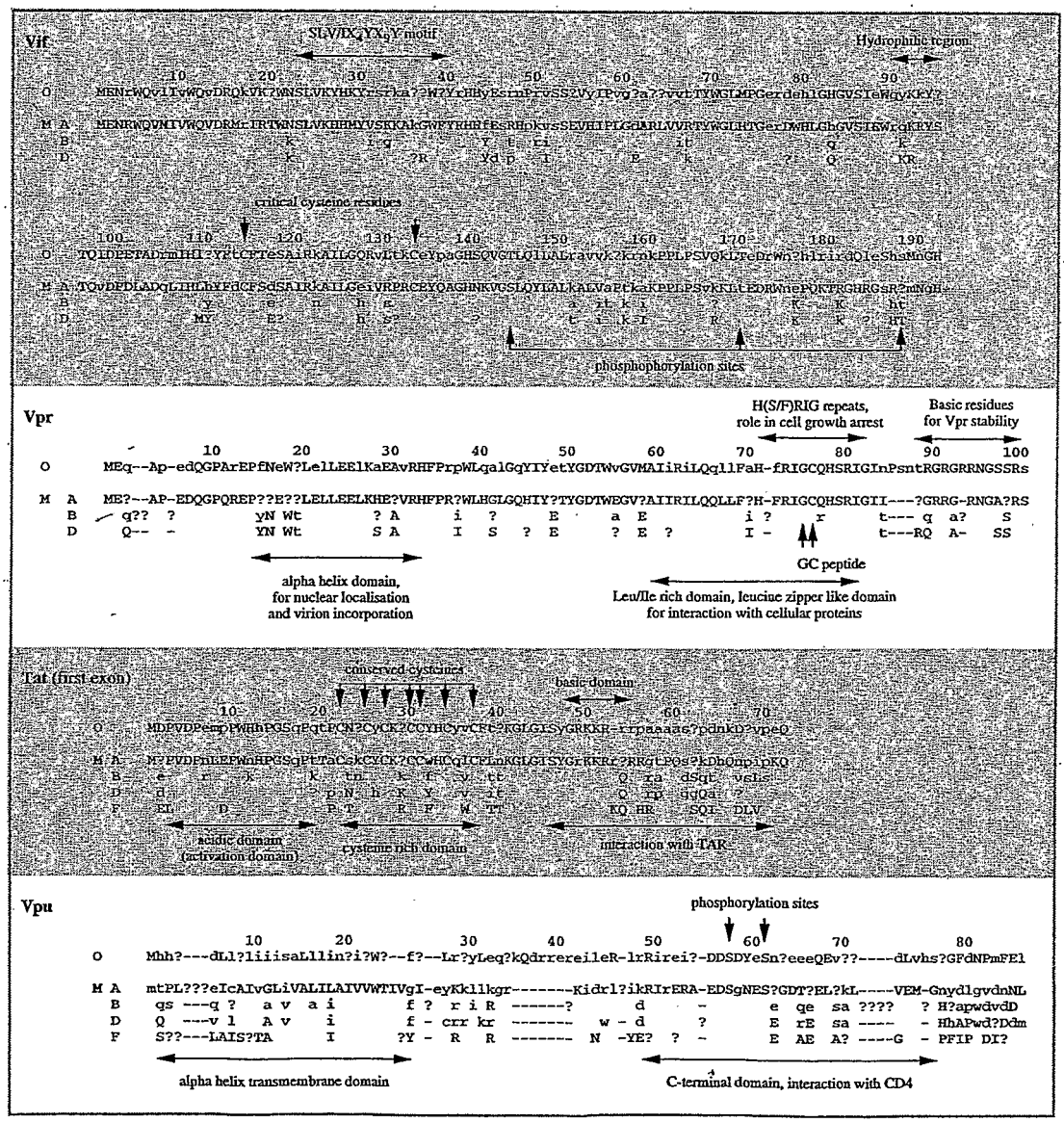


**FIG. 4.** Comparison of consensus sequences for accessory proteins for group O (Fig. 2) and M (consensus sequences for A, B, and D subtypes according to Ref. 9). See Fig. 2 caption for additional details. For each protein Vif, Vpr, Tat, and Vpu, functional domains, critical residues, specific motifs, and phosphorylation sites are indicated (see Discussion for references).

totoxic T lymphocyte pressure on the evolution of these proteins, but no observations are available concerning this. Until such data become available, the only pressure that could govern their evolution is related to their physiological properties during viral replication and conservation of the functional domains. Few data are available concerning the accessory gene sequences for the different group M genotypes, but phylogenetic analyses of the *vif-vpu* region can clearly discriminate subtypes A, B, C, D, F, and H. We have further examined whether it was possible to distinguish group O clades, using the same criteria as for group M. Our analyses (Fig. 1) indicate that at least four clusters, supported by high bootstrap values, are observed among the group O viruses; however, distances between three of them are relatively weak (Table 2), i.e., similar to intraclade distances in group M, except for the mean distance between B and D subtypes. Initial subtype classification in group M was based on partial sequences, but full-length genome sequences suggest now that B and D[79] are probably derived from a common ancestry. In a previous report,[21] phylogenetic analyses of $p24^{gag}$ (497 bp) and $C2V3^{env}$ (405 bp) sequences from seven of the strains we have studied reproducibly detected two group O clusters (BCF02 and BCF07 for the first, and MVP5180 and BCF06 for the second). Our data are in agreement with these observations, that is, these two pairs of isolates also cluster together in the accessory gene region. On the other hand, we have found that ANT-70 and BCF03 cluster together in this region despite not being significantly related on the basis of either *gag* or *env* sequence. More globally, the analyses we have performed on longer sequences (1330 bp) also fail to reveal a subtype pattern similar to that of group M viruses. On the basis of these observations, and also keeping in mind that to date, even for more distant subtypes in group M, no biological differences have been clearly demonstrated, we have decided not to assign a subtype classification for group O isolates, opting instead to await longer (full-length) sequences.

## ACKNOWLEDGMENTS

We are thankful to Beatrice Hahn for helpful comments and discussion. We also thank Florian Liegeois, Delphine Patrel, and Claire Mulanga-Kabeya for technical assistance. Project SIDAK is supported by the Agence Nationale pour les Recherches contre le SIDA (ANRS) and the French Ministry for Cooperation. This study was also supported by Grants 95017 and 95031 from the Global AIDS Program from the World Health Organization and by Contract IC18CT96-0110 from the European Community. F.B.-R. was supported by a postdoctoral fellowship from SIDACTION.

## REFERENCES

1. De Leys R, Vanderborght B, Vanden Haesevelde M, Heyndrickx, L, van Geel A, Wauters C, Bernaerts R, Saman E, Nijs P, Willems B, Taelman H, van der Groen G, Piot P, Tersmette T, Huisman JG, and Van Heuverswyn H: Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of West–Central African origin. J Virol 1990;64:1207–1216.
2. Gürtler LG, Hauser PH, Eberle J, von Brunn A, Knapp S, Zekeng L, Tsague JM, and Kaptue L: A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. J Virol 1994;68:1581–1585.
3. Vanden Haesevelde M, Decourt JL, De Leys RJ, Vanderborght B, van der Groen G, Van Heuverswijn H, and Saman E: Genomic cloning and complete sequence analysis of a highly divergent human immunodeficiency virus isolate. J Virol 1994;68:1586–1596.
4. Janssens W, Nkengasong JN, Heyndrickx L, Fransen K, Ndumbe PM, Delaporte E, Peeters M, Perret J-L, Ndoumou A, Atende C, Piot P, and van der Groen G: Further evidence of the presence of genetically aberrant strains in Cameroon and Gabon. AIDS 1994;8:1012–1013.
5. Mauclère P, Loussert-Ajaka I, Damond F, Fagot P, Souquières S, Monny Lobe M, Mbopi Keou F-X, Barré-Sinoussi F, Saragosti S, Brun-Vézinet F, and Simon F: Serological and virological characterization of HIV-1 group O infection in Cameroon. AIDS 1997;11:445–453.
6. Huet T, Cheyier R, Meyerhans A, Roelants G, and Wain-Hobson S: Genetic organisation of a chimpanzee lentivirus related to HIV-1. Nature (London) 1990;345:356–358.
7. Vanden Haesevelde M, Peeters M, Jannes G, Janssens W, van der Groen G, Sharp PM, and Saman E: Sequence analysis of a highly divergent HIV-1-related lentivirus isolated from a wild captured chimpanzee. Virology 1996;221:346–350.
8. Louwagie J, McCutchan F, Peeters M, Brenan TP, Sanders-Buell E, Eddy GA, van der Groen G, Fransen K, Gershy-Damet G-M, Deleys R, and Burke DS: Phylogenetic analysis of *gag* genes from 70 international HIV-1 isolates provides evidence of multiple genotypes. AIDS 1993;7:769–780.
9. Myers G, Korber B, Hahn BH, Jeang K-T, Mellors JW, McCutchan FE, Henderson LE, and Pavlakis GN (eds.): *Human Retroviruses and AIDS: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences 1995*. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico, 1995.
10. Birx DL, VanCott T, Michael N, McNeil J, Stamatos N, Gillian B, Davis R, Carr J, and McCutchan F: Summary of Track A: Basic science. AIDS 1996;10(Suppl. 3):S85–S106.
11. Alaeus A, Leitner T, Lidman K, and Albert J: Most HIV-1 genetic subtypes have entered Sweden. AIDS 1997;11:199–202.
12. Arnold C, Barlow KL, Parry JV, and Clewley JP: At least five HIV-1 sequence subtypes (A, B, C, D, A/E) occur in England. AIDS Res Hum Retroviruses 1995;11:427–429.
13. Lasky M, Perret JL, Peeters M, Bibollet-Ruche F, Liegeois F, Patrel D, Molinier S, Gras C, and Delaporte E: Presence of multiple non-B subtypes and divergent subtype B strains of HIV-1 in individuals infected after overseas deployment. AIDS 1997;11:43–51.
14. Soto-Ramirez LE, Renjifo B, McLane MF, Marlink R, O'Hara C, Sutthent R, Wasi C, Vithayasai P, Vithayasai V, Apichartpiyakul C, Auewarakul P, Pena Cruz V, Chui D-S, Osathanondh R, Mayer K, Lee T-H, and Essex M: HIV-1 Langerhans' cell tropism associated with heterosexual transmission of HIV. Science 1996;271:1291–1293.
15. Pope M, Frankel SS, Mascola JR, Trkola A, Isdell F, Birx DL, Burke DS, Ho DD, and Moore JP: Human immunodeficiency virus type 1 strains of subtypes B and E replicate in cutaneous dendritic cell–T-cell mixtures without displaying subtype-specific tropism. J Virol 1997;71:8001–8007.
16. Dittmar MT, Simmons G, Hibbitts S, O'Hare M, Louisirirotchanakul S, Beddows S, Weber J, Clapham PR, and Weiss RA: Langerhans cell tropism of human immunodeficiency virus type 1 subtype A through F isolates derived from different transmission groups. J Virol 1997;71:8008–8013.
17. Peeters M, Gueye A, Mboup S, Bibollet-Ruche F, Ekaza E, Mulanga C, Ouedrago R, Gandji R, Mpele P, Dibanga G, Koumare B, Saidou M, Esu-Williams E, Lombart J-P, Badombena W, LuoN, Vanden Haesevelde M, and Delaporte E: Geographic distribution of HIV-1 group O viruses in Africa. AIDS 1997;11:493–498.
18. Peeters M, Gaye A, Mboup S, Badombena W, Bassabi K, Prince-

David M, Develoux M, Liegeois F, van der Groen G, Saman E, and Delaporte E: Presence of HIV-1 group O infection in West Africa. AIDS 1996;10:343–344.
19. Hampl H, Sawitzky D, Stöffler-Meilicke M, Groh A, Schmitt M, Eberle J, and Gürtler L: First case of HIV-1 subtype O infection in Germany. Infection 1995;6:369–370.
20. Jonassen TO, Stene-Johansen K, Berg ES, Hungnes O, Lindboe CF, Froland SS, and Grinde B: Sequence analysis of HIV-1 group O from Norwegian patients infected in the 1960s. Virology 1997;231:43–47.
21. Loussert-Ajaka I, Chaix M-L, Korber B, Letourneur F, Gomas E, Allen E, Ly T-D, Brun-Vézinet F, Simon F, and Saragosti S: Variability of human immunodeficiency virus group O strains isolated from Cameroonian patients living in France. J Virol 1995;69: 5640–5649.
22. Soriano V, Guttierez M, Garcia-Lerma G, Mas A, Bravo R, Aguilera O, Perez-Labad ML, and Gonzalez-Lahoz J: First case of group O infection in Spain. Vox Sang 1996;71:66.
23. Centers for Disease Control and Prevention: Identification of HIV-1 group O infection, Los Angeles County, California. MMWR 1996;45:561–564.
24. Brenan CA, Hackett J Jr, Zekeng L, Lund J, Vallari AS, Hickman RK, Gürtler L, Kaptue L, Von Overbeck J, Hampl H, and Devare SG: Sequence of gp41$^{env}$ immunodominant region of HIV type 1 group O from West Central Africa. AIDS Res Hum Retroviruses 1997;13:901–904.
25. Hackett J Jr, Zekeng L, Brenan CA, Lund JK, Vallari AS, Hickman RK, Gürtler L, Kaptue L, and Devare SG: Genetic analysis of HIV type 1 group O p24$^{gag}$ sequences from Cameroon and Equatorial Guinea. AIDS Res Hum Retroviruses 1997;13:1155–1158.
26. Korber B, Loussert-Ajaka I, Blouin J, and Saragosti S: A comparison of HIV-1 group M and group O functional and immunogenic domains in the Gag p24 protein and the C2V3 region of the envelope protein: In: *Human Retroviruses and AIDS 1996; A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences.* Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico, 1996.
27. Balliet JW, Kolson DL, Eiger G, Kim FM, McGann KA, Srinivasan A, and Collman RG: Distinct effects in primary macrophages and lymphocytes of the human immunodeficiency virus type 1 accessory genes *vpr*, *vpu*, and *nef*: Mutational analysis of a primary HIV-1 isolate. Virology 1994;200:623–631.
28. di Marzio P, Choe S, Ebright M, Knoblauch R, and Landau NR: Mutagenic analysis of cell cycle arrest, nuclear localization, and virion packaging of HIV-1 Vpr. J Virol 1995;69:7909–7916.
29. Gonclaves J, Korin Y, Zack J, and Gabuzda D: Role of Vif in human immunodeficiency virus type 1 reverse transcription. J Virol 1996;70:8701–8709.
30. Göttlinger HG, Dorfman T, Cohen E, and Haseltine WA: Vpu protein of human immunodeficiency virus type 1 enhances the release of capsids produced by *gag* gene constructs of widely divergent retroviruses. Proc Natl Acad Sci USA 1993;90:7381–7385.
31. Harrich D, Ulich C, Garcia-Martinez LF, and Gaynor RB: Tat is required for efficient HIV-1 reverse transcription. EMBO J 1997;16:1224–1235.
32. Klimkait T, Strebel K, Hoggan M, Martin M, and Orenstein JM: The human immunodeficiency virus type 1-specific protein Vpu is required for efficient virus maturation and release. J Virol 1990;64:621–629.
33. Planelles V, Jowett JBM, Li Q-X, Xie Y, Hahn BH, and Chen ISY: Vpr-induced cell cycle arrest is conserved among primate lentiviruses. J Virol 1996;70:2519–2524.
34. Simon J and Malim MH: The human immunodeficiency virus type 1 Vif protein modulates the postpenetration stability of viral nucleoprotein complexes. J Virol 1996;70:5297–5305.
35. Vincent MJ and Abdul Jabbar M: The human immunodeficiency virus type 1 Vpu protein: A potential regulator of the proteolysis and protein transport in the mammalian secretory pathway. Virology 1995;213:639–649.
36. Yao XJ, Garzon S, Boisvert F, Haseltine WA, and Cohen EA: The effect of vpu on HIV-1-induced syncytia formation. J Acquir Immune Defic Syndr 1993;6:135–141.
37. Hamy F, Felder ER, Heizmann G, Lazdins J, Aboul-Ela F, Varani G, Karn J, and Klimkait T: An inhibitor of the Tat/TAR RNA interaction that effectively suppresses HIV-1 replication. Proc Natl Acad Sci USA 1997;94:3548–3553.
38. Inouye RT, Du B, Boldt-Houle D, Ferrante A, Park IW, Hammer SM, Duan L, Groopman JE, Pomerantz RJ, and Terwilliger EF: Potent inhibition of human immunodeficiency virus type 1 in primary T cells and alveolar macrophages by a combination anti-Rev strategy delivered in an adeno-associated virus vector. J Virol 1997;71:4071–4078.
39. Matsuda Z, Yu X, Yu QC, Lee TH, and Essex M: A virion-specific inhibitory molecule with therapeutic potential for human immunodeficiency virus type 1. Proc Natl Acad Sci USA 1993;90: 3544–3548.
40. Orsini MJ and Debouck CM: Inhibition of human immunodeficiency virus type 1 and type 2 Tat function by transdominant Tat protein localized to both the nucleus and cytoplasm. J Virol 1996;70:8055–8063.
41. Serio D, Rizvi TA, Cartas M, Kalyanaraman VS, Weber IT, Koprowski H, and Srinivasan A: Development of novel anti-HIV1 agent from within: effect of chimeric Vpr-containing protease cleavage site residues on virus replication. Proc Natl Acad Sci USA 1997;94:3346–3351.
42. Eberle J, Loussert-Ajaka I, Brust S, Zekeng L, Hauser PH, Kaptue L, Knapp S, Damond F, Saragosti S, Simon F, and Gurtler LG: Diversity of the immunodominant epitope of gp41 of HIV-1 subtype O and its validity for antibody detection. J Virol Methods 1997;67:85–91.
43. Delaporte E, Janssens W, Peeters M, Buve A, Dibanga G, Perret JL, Ditsambou V, Georges Courbot MC, Georges A, Bourgeois A, Samb B, Henzel D, Heyndrickx L, Fransen K, Van der Groen G, Larouzé B, and Mbe JR: Epidemiological and molecular characteristics of HIV infection in Gabon, 1986–1994. AIDS 1996;10:903–910.
44. Bibollet-Ruche F, Brengues C, Galat-Luong A, Galat G, Pourrut X, Vidal N, Veas F, Durand J-P, and Cuny G: Genetic diversity of simian immunodeficiency viruses from West African green monkeys: Evidence for multiple genotypes within populations from the same geographical locale. J Virol 1997;71:307–313.
45. Thompson JD, Higgins DG, and Gibson TJ: CLUSTAL W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673–4680.
46. Garnier J, Osguthorpe DJ, and Robson B: Analysis of the accuracy and implication of simple methods for predicting the secondary structure of globular proteins. J Mol Biol 1978;120:97–120.
47. Gibrat JF, Garnier J, and Robson B: Further development of protein secondary structure prediction using information theory. New parameters and consideration of residue pairs. J Mol Biol 1987;198:425–443.
48. Saitou N and Nei M: The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406–425.
49. Kimura M: *The Neutral Theory of Molecular Evolution.* Cambridge University Press, Cambridge, 1983.
50. Felsenstein J: Confidence limits on phylogenies: An approach using the bootstrap. Evolution 1985;39:783–791.
51. Felsenstein J: *PHYLIP (phylogeny interference package)*, version 3.5c. Department of Genetics, University of Washington, Seattle, Washington, 1993.

52. Tiganos E, Yao X-J, Friborg J, Daniel N, and Cohen EA: Putative α-helical structures are involved in the human immunodeficiency virus type 1 Vpu protein and CD4 are involved in binding and degradation of the Ed molecule. J Virol 1997;71:4452–4460.
53. Yao XF, Subbramanian RA, Rougeau N, Boisvert F, Bergeron D, and Cohen EA: Mutagenic analysis of human immunodeficiency virus type 1 Vpr: Role of a predicted N-terminal alpha-helical structure in Vpr nuclear localization and virion incorporation. J Virol 1995;69:7032–7044.
54. Garcia JA, Harrich D, Pearson L, Mitsuyasu R, and Gaynor RB: Functional domains required for tat-induced transcriptional activation of the HIV-1 long terminal repeat. EMBO J 1988;7:3143–3147.
55. Kuppuswamy M, Subramanian T, Srinivasan A, and Chinnadurai G: Multiple functional domains of Tat, the trans-activator of HIV-1, defined by mutational analysis, Nucleic Acids Res 1989;9: 3551–3561.
56. Mahalingam S, Patel M, Collman RG, and Srinivasan A: The carboxy-terminal domain is essential for stability and not for virion incorporation of HIV-1 Vpr into virus particles. Virology 1995;214: 647–652.
57. Oberste MS and Gonda MA: Conservation of amino-acid sequence motifs in lentivirus Vif proteins. Virus Genes 1992;6:95–102.
58. Macreadie IG, Castelli LA, Hewish DR, Kirkpatrick A, Ward AC, and Azad AA: A domain of human immunodeficiency virus type 1 Vpr containing repeated H(S/F)RIG amino acid motifs causes cell growth arrest and structural defects. Proc Natl Acad Sci USA 1995;92:2770–2774.
59. Mahalingam S, Collman RG, Patel M, Monken CE, and Srinivasan A: Role of the conserved dipeptide Gly75 and Cys76 in HIV-1 Vpr function. Virology 1995;210:495–500.
60. Wang L, Mukherjee S, Narayan O, and Zhao LJ: Characterization of a leucine-zipper-like domain in Vpr protein of human immunodeficiency virus type 1. Gene 1996;178:7–13.
61. Caputo A, Grossi MP, Bozzini R, Rossi C, Betti M, Marconi PC, Barbanti-Brodano G, and Balboni PG: Inhibition of HIV-1 replication and reactivation from latency by *tat* transdominant negative mutants in the cysteine rich region. Gene Ther 1996;3:235–245.
62. Kamine J, Elangovan B, Subramanian T, Coleman D, and Chinnadurai G: Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator. Virology 1996;216:357–366.
63. Ma X-Y, Sova P, Chao W, and Volsky DJ: Cystein residues in the Vif protein of human immunodeficiency virus type 1 are essential for viral infectivity. J Virol 1994;68:1714–1720.
64. Mousumi P and Abdul Jabbar M: Phosphorylation of both phosphoacceptor sites in the HIV-1 Vpu cytoplasmic domain is essential for Vpu-mediated ER degradation of CD4. Virology 1997;232:207–216.
65. Schubert U, Bour S, Ferrer-Montiel AV, Montal M, Maldarell F, and Strebel K: The two biological activities of human immunodeficiency virus type 1 Vpu protein involve two separable structural domains. J Virol 1996;70:809–819.
66. Hillman K, Qian J, Siegel JN, Roderiquez G, Blackburn R, Manischewitz J, Norcross M, and Golding H: Reduced susceptibility of HIV-1 infection of ethyl-methanesulfonate-treated CEM subclones correlates with a blocade in their protein kinase C signaling pathway. J Immunol 1992;148:3991–3998.
67. Jokobovits A, Rosenthal A, and Capon DJ: Trans-activation of HIV-1 LTR-directed gene expression by *tat* requires protein kinase C. EMBO J 1990;9:1165–1170.
68. Qatsha KA, Rudolph C, Marne D, Schachtele C, and May WS: Go 6976, a selective inhibitor of protein kinase C, is a potent antagonist of human immunodeficiency virus type 1 induction from latent/low-level-producing reservoir cells in vitro. Proc Natl Acad Sci USA 1993;90:4674–4678.
69. Yang X, Gonclaves J, and Gabuzda D: Phosphorylation of Vif and its role in HIV-1 replication. J Biol Chem 1996;271:10121–10129.
70. Braaten D, Franke EK, and Luban J: Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIVcpzGAB but not group O HIV-1 or other primate immunodeficiency viruses. J Virol 1996;70:4220–4227.
71. Franke EK, Yuan HE, and Luban J: Specific incorporation of cyclophilin A into HIV-1 virions. Nature (London) 1994;372: 359–362.
72. Colgan J, Yuan HE, Franke EK, and Luban J: Binding of the human immunodeficiency virus type 1 Gag polyprotein to cyclophilin A is mediated by the central region of capsid and requires Gag dimerization. J Virol 1996;70:4299–4310.
73. Billich A, Hammerschmid F, Peichl P, Wenger R, Zenke G, Quesniaux V, and Rosenwirth B: Mode of action of SDZ NIM 811, a nonimmunosuppressive cyclosporin A analog with activity against human immunodeficiency virus (HIV) type 1: Interference with HIV protein–cyclophilin A interactions. J Virol 1995;69:2451–2461.
74. Camaur D and Trono D: Characterization of human immunodeficiency virus type 1 Vif particle incorporation. J Virol 1996;70: 6106–6111.
75. Simon JH, Fouchier RAM, Southerling TE, Guerra CB, Grant CK, and Malim MH: The Vif and Gag proteins of human immunodeficiency virus type 1 colocalize in infected human T cells. J Virol 1997;71:5259–5267.
76. Ewart GD, Sutherland T, Gage PW, and Cox GB: The Vpu protein of human immunodeficiency virus type 1 forms cation-selective ion channels. J Virol 1996;70:7108–7115.
77. Chen BK, Gandhi RT, and Baltimore D: CD4 down-modulation during infection of human T cells with human immunodeficiency virus type 1 involves independent activities of Vpu, Env, and Nef. J Virol 1996;70:6044–6053.
78. Piller SC, Ewart GD, Premkumar A, Cox GB, and Gage PW: Vpr protein of human immunodeficiency virus type 1 forms cation-selective channels in planar lipid bilayers. Proc Natl Acad Sci USA 1996;93:111–115.
79. Cornelissen M, van den Burg R, Zorgdrager F, Lukashov V, and Goudsmit J: *pol* gene diversity of five human immunodeficiency virus type 1 subtypes: Evidence for naturally occurring mutations that contribute to drug resistance, limited recombination patterns, and common ancestry for subtypes B and D. J Virol 1997; 71:6348–6358.

Address reprint requests to:
*Martine Peeters*
*ORSTOM, Laboratoire Rétrovirus*
*911, Avenue Agropolis*
*BP 5045*
*34032 Montpellier cedex 1, France*

*E-mail: martine.peeters@mpl.orstom.fr*

Volume 14 Number 11 July 20, 1998

ISSN: 0889-2229

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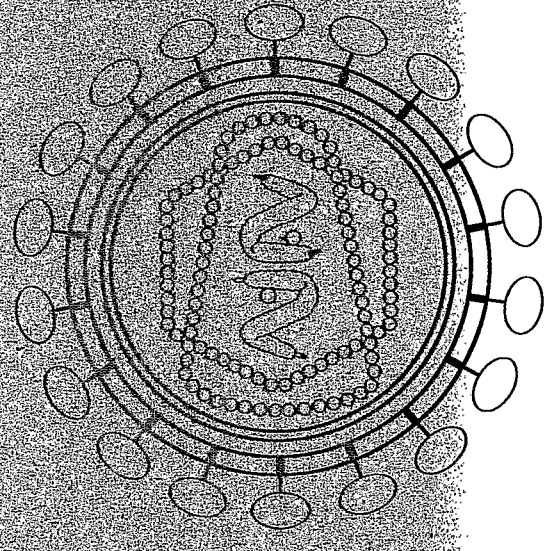



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