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Short Communication

Molecular Characterization of the Envelope Transmembrane Glycoprotein of 13 New Human Immunodeficiency Virus Type 1 Group O Strains from Six Different African Countries

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GENETIC ANALYSIS OF MANY HIV-1 isolates collected from different parts of the world revealed that they can be divided into two major phylogenetic groups, group M and group O. HIV-1 group M isolates can be further subdivided into at least 10 different genetic subtypes and group M infections predominate worldwide.[1] The first HIV-1 group O viruses to be described were the ANT-70 and MVP-5180 strains, both isolated from Cameroonians.[2,3] HIV-1 group O seems to be endemic in West Central Africa, especially Cameroon, where the frequency of infection is estimated to be 2–5% of HIV-1-infected individuals, but sporadic cases have been documented in West, East, and southern Africa.[4,5] HIV-1 group O-infected individuals have also been identified in Europe (Belgium, France, Germany, Spain) and in the United States.[2,6–9] With the exception of a French woman,[10] all reported group O-infected individuals originate from or have a connection to West Central Africa. In addition, the first case of AIDS documented in Europe was a Norwegian sailor infected with a group O virus.[11] Infections with HIV-1 isolates from group O present a public health problem because antibodies against them might not be detected. Indeed, several reports indicated that selected commercial assays failed to detect or only weakly detected group O sera.[12,13] Since the publication of these observations, screening tests have been adapted to detect antibodies against these divergent strains, either by using broadly cross-reactive gp41 group M peptides or by including specific group O peptides from this region. However, identification and monitoring of HIV variants are essential for maintaining the sensitivity of current screening assays. We report here on the genetic characterization of the immunodominant region from gp41 for 13 new HIV-1 group O samples from 6 different African countries.

Patients were identified as being infected with an HIV-1 group O virus, using a specific serological testing algorithm as previously described.[4] Briefly, sera were tested by enzyme-linked immunosorbent assay (ELISA), using a combination of V3 peptides from ANT-70 and MVP-5180, and all reactive samples were retested in a line immunoassay based on V3 peptides from different M and O strains (consensus M, M-Mal, O-ANT-70, O-VI686, and O-MVP5180; Research Products, Innogenetics, Ghent, Belgium). Among the 13 samples that were further studied genetically, 7 were from Cameroon (95CA-BSD189, 95CA-BSD422, 95CA-BSD649, 95CA-MP340, 95CA-MP95B, 95CA-MP448, and 95CA-MP450), 2 were from Gabon (95GA-189 and 95GA-533), and the others were from Chad (95TD-320), Nigeria (96NG-KGT008), Senegal (95SN-MP331), and Niger (95NE-772P94). For five samples DNA was extracted from primary or cultured peripheral blood mononuclear cells (PBMCs) using the IsoQuick isolation kit (Microprobe, Garden Grove, CA). For the remaining samples viral RNA was extracted from sera and reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously,[4] using 0.5 μM reverse primer (41-4). A nested PCR was used to amplify a fragment of approximately 420 bp. Outer primers allow amplification of HIV-1 from groups O and M

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(sense 41-1, 5′-GGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCG-3′; antisense 41-4, 5′-TCTGAAACGACAGAGGTGAGTATCCCTGCCTAA-3′) and inner primers were determined according to the HIV-1-ANT70 sequence (sense 41-6, 5′-TGGATCCCACAGTGTACTGAAGGGTATAGTGCA-3′; antisense 41-7, 5′-CATTTAGTTATGTCAAGCCAATTCCAAA-3′). PCRs were performed in a final volume of 100 μl 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 μM concentration of each primer. After an initial denaturation step of 3 min at 94°C, 35 cycles were performed at 94°C for 20 sec, 50°C for 30 sec, and 72°C for 1 min, followed by a final extension for 7 min. For the second round, 5 μl from the first amplification was subjected to the same cycling conditions. Both strands of each fragment were sequenced, using an Applied Biosystems sequencer (model 373A; Applied Biosystems/Perkin-Elmer, Foster City, CA) and a dideoxy terminator procedure, as specified by the manufacturer. Direct sequencing of the PCR products was performed and ambiguities observed at a limited number of positions in some sequences were resolved when joining the overlapping fragments.

The length of the sequenced fragment ranges from 330 to 351 bp owing to insertions and deletions. Phylogenetic trees were constructed from nucleotide sequences (final alignment,

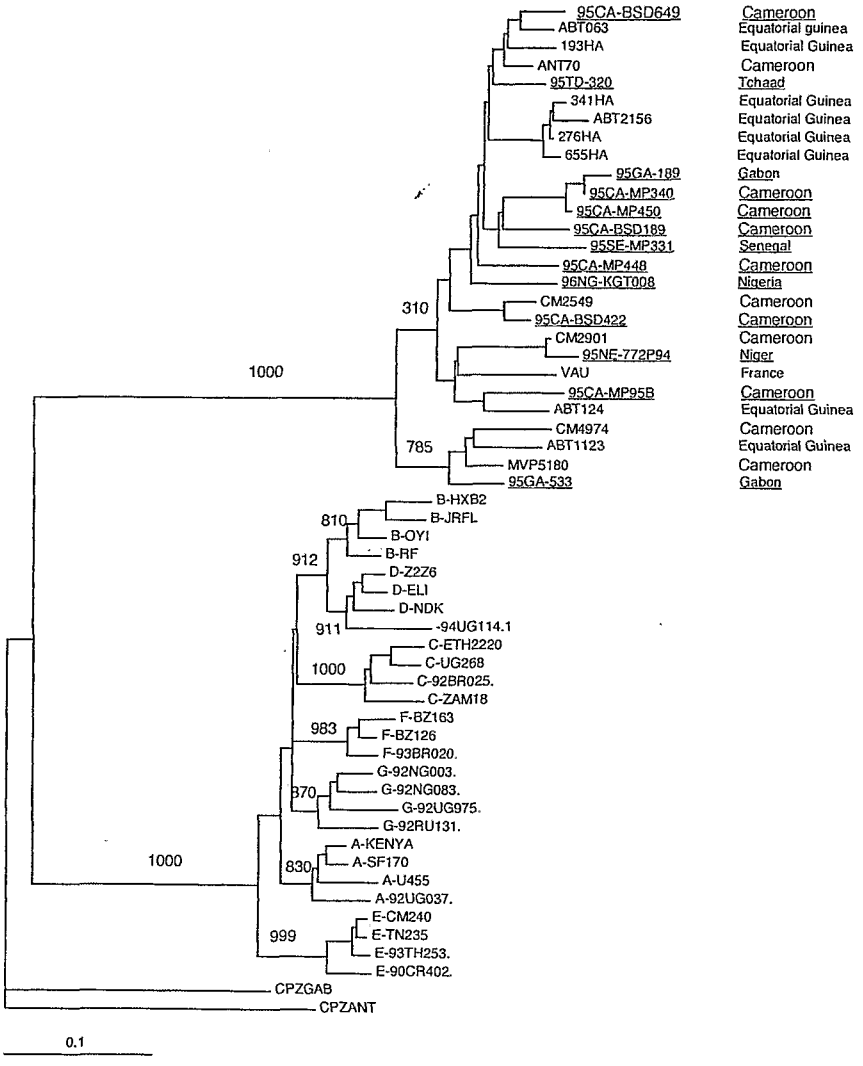

FIG. 1. Phylogenetic tree analyses for the sequenced region of the transmembrane glycoprotein for HIV-1 group O and M strains. The viruses characterized in this study are underlined. Phylogenetic relationships were determined by the neighbor-joining method and implemented using CLUSTAL W. A total of 301 sites was analyzed (gaps deleted from nucleotide alignment). The numbers given at the branch points represent bootstrap values out of 1000 obtained for the neighbor-joining methods. The sequences have been submitted to the EMBL database under accession numbers Y09767 (95NE-772P94), Y09768 (95CA-MP448), Y09769 (95CA-BSD649), Y09770 (95GA-533), Y09771 (95CA-MP95B), Y09772 (95GA-189), Y09773 (95CA-MP450), Y09775 (95TD-320), Y09776 (95CA-BSD189), Y09777 (96NG-KGT008), Y09778 (95CA-BSD422), Y09779 [95SE-MP331 (FABA)], and Y09780 (95CA-MP340).

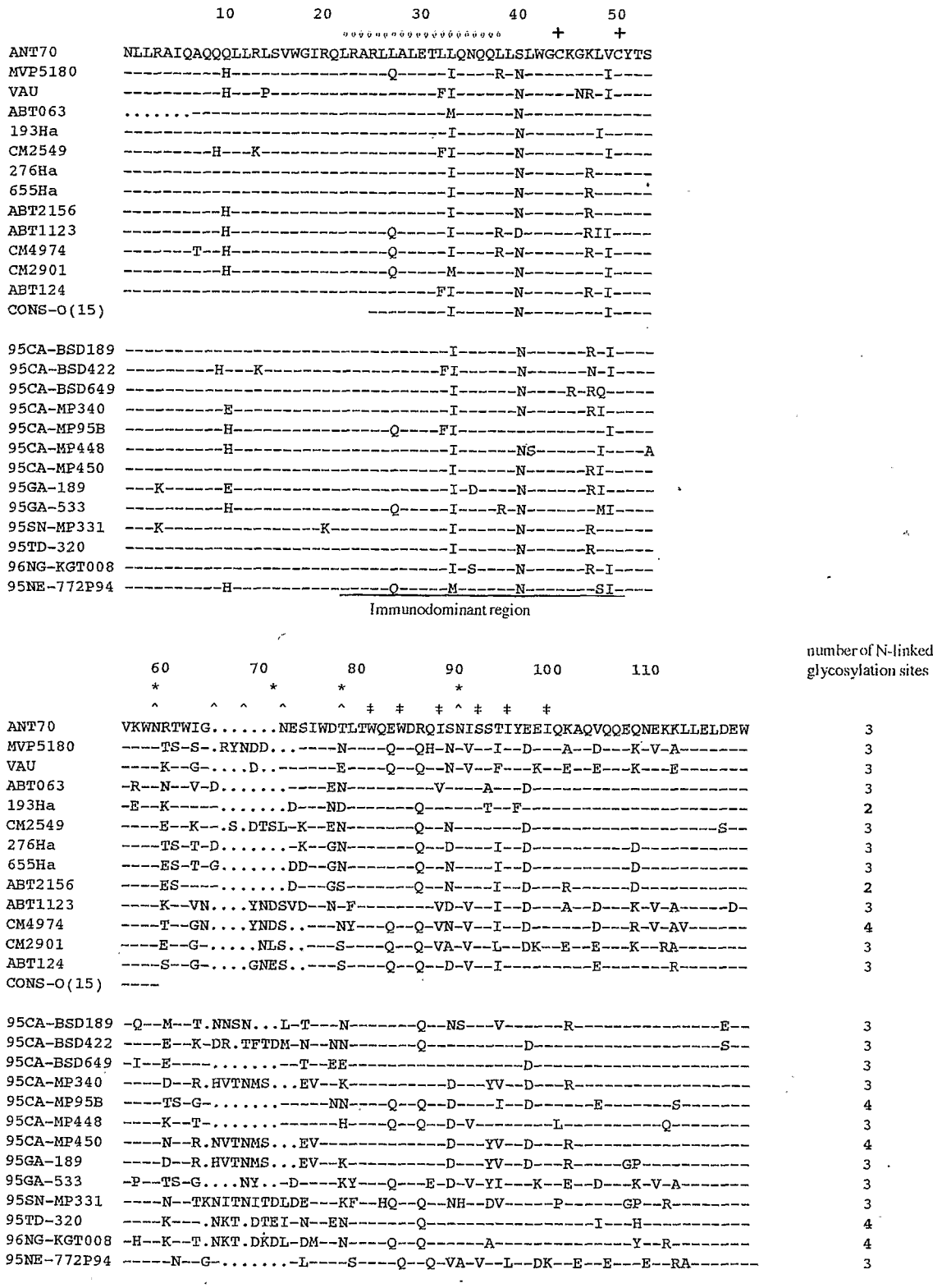

FIG. 2. Alignment of the deduced amino acid sequences of group O strains compared with previously published sequences.[2,3,10,14–16] Dots (.) represent gaps introduced to maximize alignment and dashes (–) represent identical amino acids. The immunodominant region is underlined; within this domain a diamond (◇) indicates the immunosuppressive peptide (ISU-peptide) by analogy to HIV-1 group M viruses, and a plus (+) indicates the two conserved cysteine residues flanking the principal immunodominant domain (PID). The potential N-linked glycosylation sites [N-X-(T or S)], which are shown by carets (^) above the asparagine residues, are indicated to the right of the sequences. The positions of the N-linked glycosylation sites in group M sequences are indicated by asterisks (*). A double dagger (‡) refers to conserved residues involved in cell fusion entry.

301 bp, after exclusion of the gaps) by the neighbor-joining and maximum-parsimony methods, with the new sequences described here and group O sequences from the database together with reference strains from group M representing subtypes A to G. Consistent results were obtained by the two methods. The phylogenetic analysis showed that all our samples belonged to group O, but in contrast to group M viruses no subtypes could be identified (Fig. 1). Within group O, the samples can be divided into those that branch with ANT-70, VAU, or MVP-5180, although these subgroups were not supported by high bootstrap values. The majority of the samples branch with the ANT-70 strain. The evolutionary distance matrix, calculated using DNAdist (Kimura two-parameter method) on the same strains, and by using the same matrix as for the phylogenetic analysis, shows that group O isolates can be as closely related to each other as are isolates within a group M subtype, and as distantly related as observed between group M subtypes. The mean distance among the group M strains was 14% (ranging from 1.7% to 23%), with an intrasubtype distance ranging from 3.7 to 7% and an intersubtype distance ranging from 10 to 19%. The mean distance among group O strains was also 14% and ranged from 1.2 to 24%.

Figure 2 shows the alignment of the protein sequence from the gp41 region that was PCR amplified. The sequenced region includes the immunodominant region (indicated in Fig. 2), which can be divided into an immunosuppressive (ISU) peptide of 17 amino acids and an immunodominant cysteine loop. Within group O isolates, the immunodominant region is relatively conserved, when comparing our sequences and previously published sequences, but group O isolates are quite divergent from the group M subtype B isolates, which may explain the less efficient detection of group O infection by immunoassays based on group M subtype B reagents.[14–16]

Among group M viruses, four N-linked glycosylation sites are conserved in the gp41 extracellular domain, and they have been shown to play important roles in the extracellular processing of gp160[17,18] and in the fusogenic properties of gp41.[19,20] Most of the group O viruses described here (9 of 13) contain only 3 potential N-linked glycosylation sites, whereas only 4 strains contain 4 sites. Among the already published strains, two have only two N-linked glycosylation sites. Only the position of the first site is highly conserved (position 60 in the alignment) and the positions of the three other sites vary among the strains, in contrast to group M viruses, in which these sites are conserved at fixed positions. Differences in the N-linked glycosylation patterns between groups M and O have also been reported for the C2V3 region of the envelope.[6] The differences observed between the two HIV-1 groups in the gp41 region raise the possibility that these viruses use different mechanisms, for example, in the extracellular processing of the envelope precursor or in the domains involved in membrane fusion. Finally, the sequenced region also includes critical residues that are involved in cell fusion in group M strains. These residues are also highly conserved in group O and include W81, W84, I88, S92 (instead of T in group M), I95, and I99.[21]

The immunodominant region of gp41 plays a central role in the identification of antibody against HIV-1. Detection of all HIV-infected individuals continues to be one of the major goals to prevent the spread of HIV infection. Therefore sequence information on the gp41 immunodominant region among group O isolates is of major interest, especially in the design of future serologic assays.

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