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Sequence Note

vpu and *env* Sequence Variability of HIV-1 Isolates from Tanzania

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THE NUCLEOTIDE SEQUENCES of an approximately 1400-bp region spanning the *vpu* and *env* (V1–V3) genes of nine HIV-1 isolates originating from Tanzania have been determined. Peripheral blood lymphocyte (PBL) specimens were obtained in 1988 from urban patients with clinical signs of AIDS attending the Muhimbili Medical Centre (Dar es Salaam, Tanzania).[1] Nine samples (TZ005, TZ012, TZ016, TZ017, TZ023, TZ030, TZ053, TZ064, and TZ112) were randomly chosen for virus isolation by cocultivation with HIV-negative donor PBLs. Viral DNA sequences between the positions 5543 and 6956 (according to the HIV-1 Lai[2] sequence) were amplified by polymerase chain reaction (PCR), using two sets of primer pairs, subcloned into a Bluescript vector, and sequenced on both strands. In addition, the V3 sequence of a tenth isolate (TZ080) was determined. Sequence analysis revealed *vpu* and *env* open reading frames (ORFs) for all clones, except two that had a missense mutation in *vpu* (TZ016) or *env* (TZ017). Accession numbers for the sequences have been assigned in the GenBank Database (U12406–U12415).

The Vpu sequences examined showed a high degree of homology among all isolates, with TZ005, TZ016, and TZ030 having identical sequences. The phosphorylation sites (S^{52} and S^{56}) joining two amphipathic α helices were completely conserved.[3] In isolate TZ064 the *vpu* ORF started with an AUA instead of an AUG, similar to the *vpu* sequence of HIV MAL.[4]

Phylogenetic tree analysis indicated that most of our isolates fall into the D subtype. Exceptions are TZ016 and TZ017, which are closer to the A subtype than to other HIV-1 subtypes (Fig. 1). The analysis of the deduced protein sequences of the V3 loop, which contains the principal neutralizing domain (PND), revealed an amino acid pattern closely related, but not identical, to known African HIV isolates (Fig. 2). While the fourth position of the PND was invariantly a Q, the second position was more heterogeneous and was occupied by either S, P, L, T, I, or Q. The GSGQ motif was found in four isolates (TZ005, TZ030, TZ053, and TZ080), and the GPGQ motif was

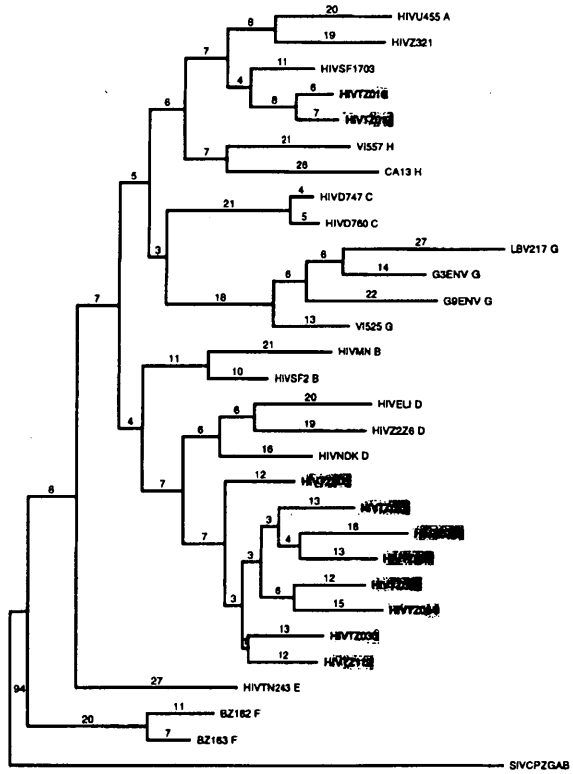

FIG. 1. Phylogenetic tree analysis based on 231 sites (184 varied sites) from the V3 region of HIV-1 *env* sequences. The 10 Tanzanian (TZ) sequences discussed in this article are highlighted, 8 clustering with subtype D sequences and 2 clustering with subtype A sequences. The tree was constructed using the PAUP parsimony program. All sequences including the Tanzanian sequences are available through GenBank and the HIV Sequence Database.

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A

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TZ005          CTRPYR NTRQRTHIGSGQALYTTRITG GDIRRAHC
TZ012          -S---Y -Q-R-----T---------R  ----Q---
TZ016          -S--N N---KSVR--P---F-A- GDII----Q---
TZ017          ----S N---KSVR--P---F-A- GDII----Q---
TZ023          ------ ---R--P--L-------N-NIL------Y-
TZ030          ----Y K--I------S----F---G  I--P---Y-
TZ053          -S---IK-I-R---------Y---NIG  -T--P---
TZ064          ------ SSTR--P--I--S----K N-M----Q---
TZ080          -----HKD-I-R-------------L  K----Q-Y-
TZ112          ----F TR-----S--Q---F-RA TRII----Q-Y-
TZ cons.       CTRPY  NTRRRTHIGSGQALYTTRIDIIGDIRQAHC
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B

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CONSENSUS      CTRP NNNTRKSIHIGPGQAFYATG DIIGDIRQAHC
A cons.        ---- -------V---------- ----------
B cons.        ---- ----------R---T-- E----------
C cons.        ---- --------R-----T----- ----------
D cons.        ----Y ----QRT-------L-T-  R----------
E cons.        ---- S----T--T-----V--R-- -------K-Y-
F cons.        ---- ---------L---------- -------K---
TAN cons.[5]   ----Y ----Q-T-------L-T-  ----------
TZ cons.       ----Y  ---RRT---S-Q-L-T-RI----------
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FIG. 2. HIV-1 V3 loop sequences. (A) V3 loop sequences of Tanzanian isolates described in this study. (B) Comparison of HIV-1 subtype V3 consensus sequences derived from Myers *et al.*[2] with V3 consensus sequences of Tanzanian HIV-1 isolates described by Zwart *et al.*[5] and in this study. Multiple sequences were aligned using the MegAline program (Clustal method) of the DNASTAR software.

found in two cases (TZ016 and TZ017). Interestingly, the V3 variability of the HIV isolates described here was greater than previously reported for Tanzanian viruses, in which the GPGQ motif was thought to be a consensus sequence.[5] All except two isolates (TZ016 and TZ017) had an R instead of a S at position 11 of the V3 loop. In addition, isolates TZ012 and TZ112 had an R instead of a D at position 25. Both substitutions are characteristic for subtype D sequences.[2] It had been described previously that these changes may result in a syncytium-inducing, nonmonocytotropic phenotype.[6,7] The two glycosylation sites at the N and C termini of the V3 loop were conserved in most of our Tanzanian isolates; only TZ005 lacked the C-terminal site. In contrast, the second N-linked glycosylation site of the V3 loop was only weakly conserved, and was not present in isolates TZ064, TZ080, and TZ112.

Although AIDS viruses are believed to have originated from Africa, little is known about the sequence variability of African HIV-1 isolates, compared to the information available on European viruses. The results presented here on the variability of East African HIV-1 isolates are consistent with the view that these are rapidly changing viruses for which further variants are likely to be discovered.

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