JOURNAL OF VIROLOGY, July 1992, p. 4546–4550
0022-538X/92/074546-05$02.00/0

Vol. 66, No. 7

In Vivo Genetic Variability of the Human Immunodeficiency Virus Type 2 V3 Region

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Received 16 January 1992/Accepted 6 April 1992

The principal neutralizing epitope of the human immunodeficiency virus type 1 (HIV-1) lies between two invariant cysteines in the third variable region (V3) of the viral envelope (gp120), and its amino acid sequence varies among different HIV-1 isolates. HIV-2 carries an analogous amino acid sequence between two cysteines of the V3 regions, but its functional similarity with the HIV-1 principal neutralizing epitope is uncertain. We studied the degree of genetic variation of the HIV-2 V3 region in fresh blood samples from 12 HIV-2-seropositive individuals from Guinea-Bissau. Polymerase chain reaction was used to amplify viral fragments of 465 bp containing the V3 region from cellular DNA. Nucleotide sequence analysis of the entire envelope fragment from each patient revealed that the degree of variation among field isolates of HIV-2 is comparable to that observed in the analogous region of HIV-1. Most of the HIV-2 isolates studied were highly related, suggesting the existence of a limited number of different viral strains in the cohort studied. Thus, the HIV-2 and HIV-1 V3 regions vary to a similar degree and may also have analogous functions.

Human immunodeficiency virus type 2 (HIV-2) was discovered in and isolated mainly from patients of West African origin (2, 7, 41). HIV-2 causes AIDS in humans but is far less pathogenic than HIV-1 (29). HIV-2 is more closely related both immunologically and genetically (6, 11, 12, 17, 20) to simian immunodeficiency virus (SIV_{mac}, SIV_{mne}, or SIV_{sm}) than to HIV-1. Several lines of evidence suggest that HIV-2 might have been transmitted horizontally from monkeys to humans (21, 30). Unlike HIV-1, HIV-2 can infect macaques and baboons; as these animals do not develop clinical immunodeficiency (10, 13, 34, 38), they can be used to test the protective effect of a vaccine against viral infection. However, the usefulness of HIV-2/SIV in monkeys as a model for HIV-1 infection of humans has been questioned because of the apparent biological-pathologic difference between these virus groups. In particular, the failure (39) or the low efficiency (3) of HIV-2/SIV linear V3 region peptides to induce neutralizing antibodies compared with that of HIV-1 (16, 31, 36, 40) suggests that an additional region(s) of the HIV-2/SIV envelope protein participates in neutralization. In HIV-1, the principal neutralizing domain is in the V3 region, which lies between two disulfide bonds thought to confer a loop structure (V3 loop) (28) and is subjected to a high degree of genetic variation (18). In contrast, for SIV, low variability within the V3 region has been reported (4, 5, 23, 35). The origin of the SIV family of viruses (SIV_{mac}, SIV_{mne}, and SIV_{sm}) is unknown, but they possibly represent a discrete viral population that entered captive colonies in the United States (8); if so, the degree of variability of the SIV V3 region measured in these sibling viruses should be low.

To investigate the spectrum of HIV-2 variability in nature, we analyzed viral sequences in DNA obtained from the fresh peripheral blood of healthy HIV-2-seropositive Guinea-Bis-

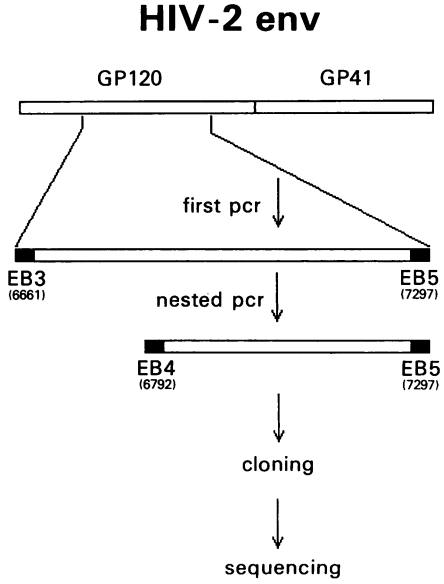

FIG. 1. Schematic representation of the polymerase chain reaction strategy employed. Sequences of the primers used were as follows: for EB3, 5′ TTTGAGCGGCCGCGGTACTCAAAAGATG TGG sense (6661 to 6677); for EB4, 5′ TTTGAGCGGCCGGGATA GTGTGCACCACCGGG sense (6792 to 6809); for EB5, 5′ ACTTA GAATTCTCCTCTGCAGTTAGTCCAC antisense (7279 to 7297). The conditions for polymerase chain reaction analysis were as follows. DNA (0.1 to 1 μg) was amplified with *Taq* polymerase (Perkin-Elmer) with the reagents provided by the manufacturer. Initial denaturation was at 94°C for 5 min; initial denaturation was followed by 35 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 58°C, and 1 min of elongation at 72°C. Final elongation was for 10 min at 72°C.

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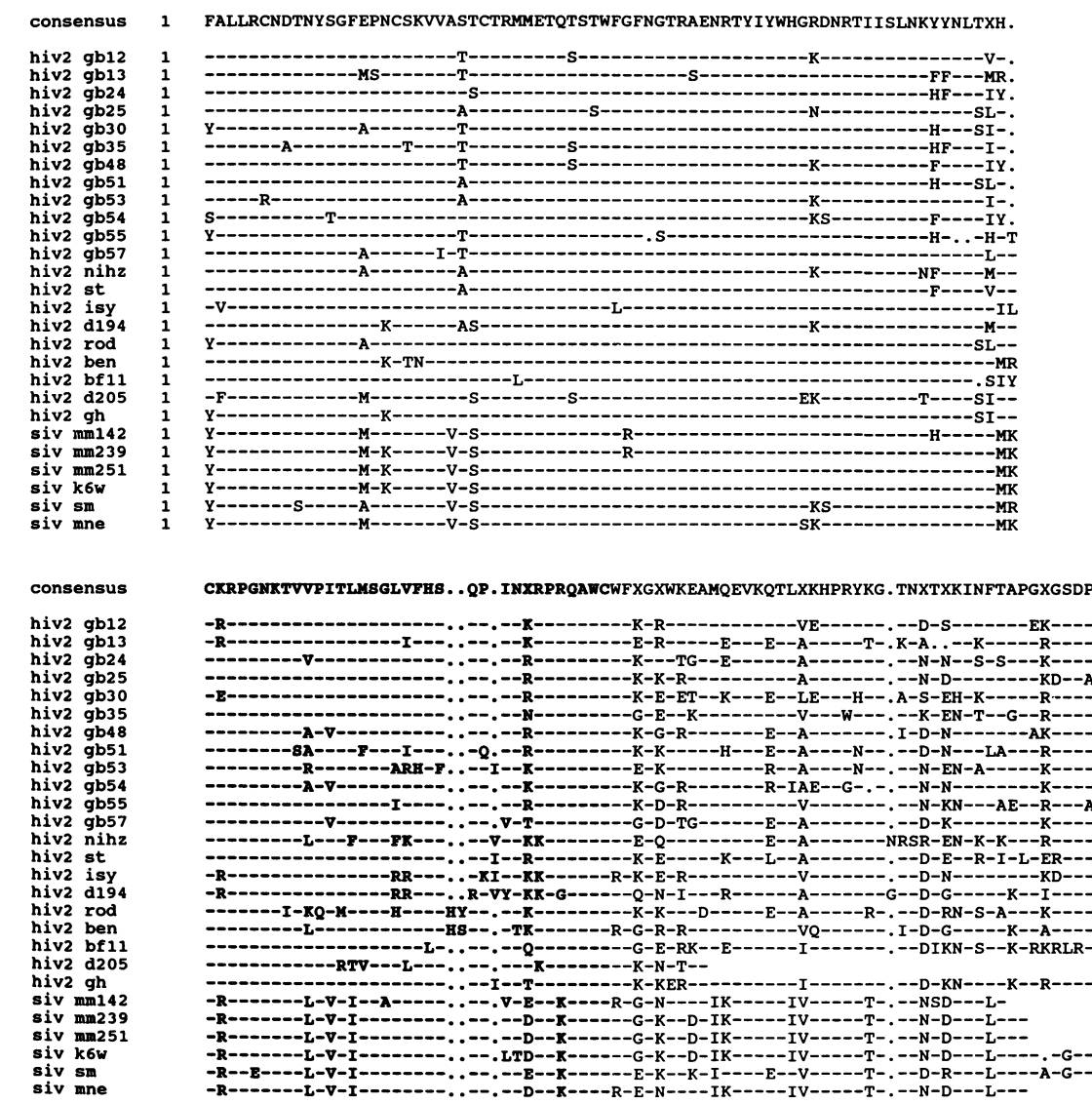

FIG. 2. Amino acid sequence alignment of the entire HIV-2/SIV envelope sequences studied. Number 1 corresponds to amino acid residue 223 in the envelope sequence of HIV-$2_{SBL/ISY}$ (11). The letters represent the single-letter amino acid code, with the exception of X, which indicates the lack of a predominant amino acid in this position. The dashed lines represent identity of the amino acid with the consensus sequence, whereas the dots represent amino acid deletions. The V3 region is depicted with boldface letters.

sau blood donors. As schematically represented in Fig. 1, two oligonucleotide primers (EB3 and EB5), chosen according to the alignment of three known viral isolates (HIV-2_{ISY} [11], HIV-2_{NIH-Z} [41], and HIV-2_{ROD} [17]), were used to amplify a fragment of 638 bp encompassing the HIV-2 V3 region. The sensitivity of the polymerase chain reaction was increased by a second amplification of the polymerase chain reaction product by using EB5 in conjunction with a third internal primer, EB4. The amplified viral DNA fragments were cloned, and the DNA sequences obtained were subjected to computer analysis (Microgenie; Beckman).

Alignments of the predicted envelope amino acid sequences of the HIV-2 studied, the other known HIV-2 isolates (9, 11, 17, 19, 24–26, 41), and SIV_{mac}, SIV_{sm}, and SIV_{mne} (6, 12, 20, 21, 33) are presented in Fig. 2. The overall alignment of the amino acid sequences indicated the presence of gaps due to the insertion of six nucleotides in HIV-2_{ROD} and HIV-2_{BEN} and of three nucleotides in HIV-2_{NIH-Z}, HIV-2_{ISY}, HIV-2_{D194}, HIV-2_{ST}, and HIV-2_{GH} and HIV-2_{GB53} from the Guinea-Bissau cohort. The highest degree of amino acid conservation was observed at the amino terminus of the envelope region studied. Immediately before the first cysteine of the V3 region, a stretch of seven amino acids showed high variability and most of the amino acid changes were nonconservative. In contrast, the same amino acid region among the SIV isolates was well conserved. In HIV-1, the V3 loop crown region (GPGR) is highly conserved among different viruses (27), and a region with a different amino acid composition (FHSQ) in the middle portion of the V3 region is also highly conserved among the HIV-2/SIV isolates (14). In HIV-2, the amino (RPGNKT) and carboxyl (QAWC) terminus regions adjacent to the cysteines, which delimit the V3 region, are also highly conserved. The highest variability in both nucleotide

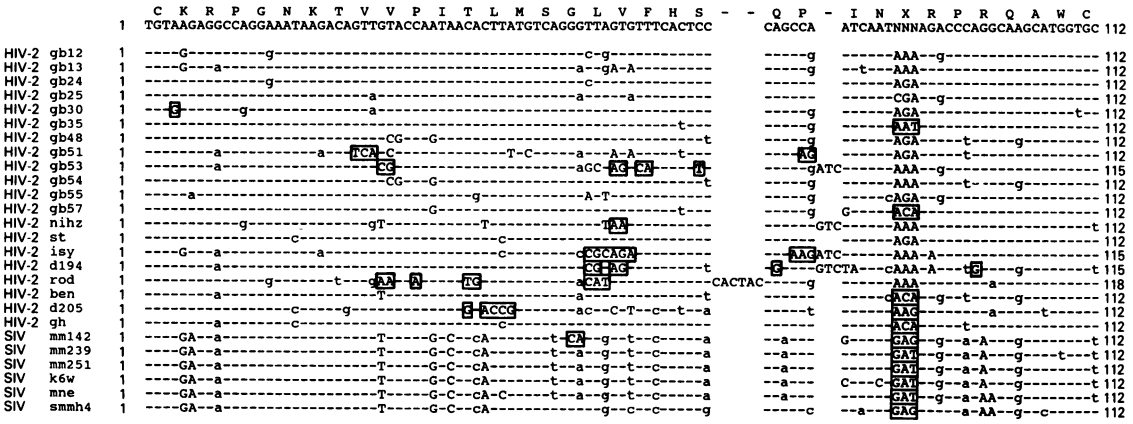

FIG. 3. Amino acid and nucleotide sequence alignment of the HIV-2/SIV V3 region (cysteine to cysteine). The upper lines represent the consensus amino acid and nucleotide sequences, and number 1 corresponds to nucleotide position 7030 of the HIV-$2_{SBL/ISY}$ sequence (11). Capital letters represent nucleotide substitutions that yielded conservative amino acid changes. The boxed capital letters represent substitutions that yielded nonconservative amino acid changes. Lowercase letters represent silent nucleotide changes.

and amino acid sequences was found at the 3′ end of the V3 region, as in HIV-1.

Detailed analysis of the qualitative nucleotide changes within the V3 region (Fig. 3) indicated that more than one-third of the nucleotide substitutions changed the amino acid sequence and that one-half of the amino acid changes were nonconservative. Thus, this region of HIV-2, the equivalent of which has been shown for HIV-1 to be important in neutralization (16, 31, 36, 40) as well as in determination of viral tropism (2), displayed high variability in vivo.

To better quantitate the degree of genetic changes within the HIV-2 V3 region, we compared several previously published HIV-2 DNA sequences (9, 11, 17, 19, 24–26, 41) with those obtained from the Guinea-Bissau cohort studied and the known SIV isolates (6, 12, 20, 21, 32). The average divergence at both the nucleotide and amino acid levels of the Guinea-Bissau HIV-2 isolates was twice that of SIV and approximately one-half of the genetic variability observed among the other known HIV-2 isolates (Fig. 4A). These findings are indicative of geographical variation of HIV-2 viral strains and clustering within the community studied.

High genetic variability of the V3 region has been shown among several HIV-1 isolates (27). To ascertain whether the HIV-1 and HIV-2 V3 regions varied to a comparable degree, we compared sequences for each member of the two virus families with the nucleotide consensus sequence of this region for each family. The comparison included a total of 22 HIV-2 and 245 HIV-1 samples (27). The results, graphically represented in Fig. 4B, showed an overall comparable degree of genetic variation (around 14%) among the HIV-2 and HIV-1 isolates studied.

To better evaluate the existence of geographical clusters of HIV-2 isolates, we constructed a dendrogram from the V3 region DNA sequences of the known HIV-2 isolates, SIV isolates, and Guinea-Bissau cohort HIV-2 isolates (Fig. 5). The SIV isolates clustered with each other and separately from the HIV-2 isolates from Guinea-Bissau (10 of 12). A subcluster of HIV-2 isolates was observed to contain HIV-

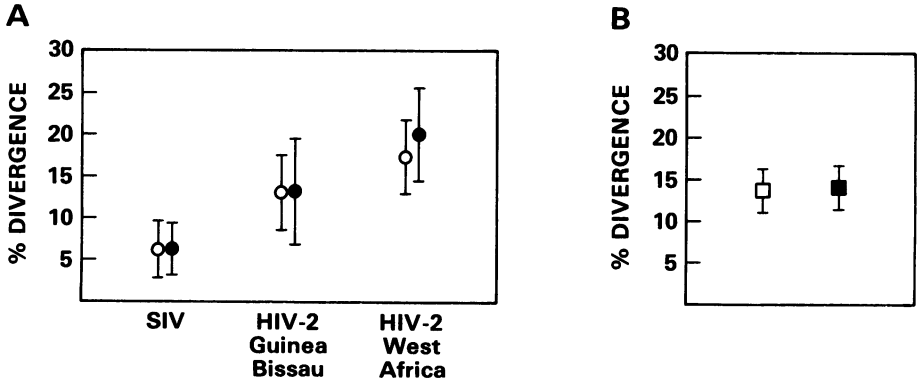

FIG. 4. Diagram of the percent divergence among HIV-1/HIV-2/SIV V3 regions (cysteine to cysteine). (A) Presented on the *y* axis is the percent divergence in the V3 regions. The open circles represent nucleotides and the closed circles represent amino acids. The SIV group includes SIV_{mac}, SIV_{mne}, and SIV_{sm} (6, 12, 20, 21, 32). The HIV-2 Guinea-Bissau group includes the 12 viruses studied as described in this report. HIV-2 West Africa represents all the HIV-2 sequences known plus two of the Guinea-Bissau viruses (GB55, GB57) (9, 11, 17, 19, 24–26, 41). (B) Presented on the *y* axis is the percent nucleotide divergence in the V3 region. The open square represents the value obtained by comparison of the known HIV-2 sequences and the samples studied as described in this report, and the closed square represents all the HIV-1 samples studied as described in reference 27.

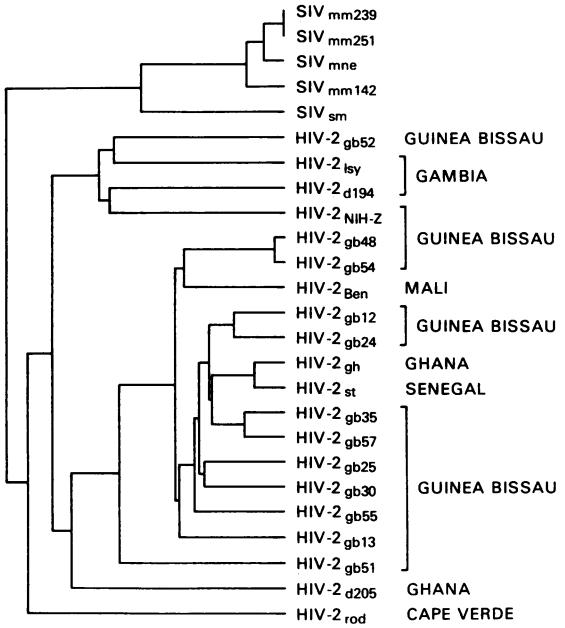

FIG. 5. Cluster analysis of the V3 region (cysteine to cysteine) DNA sequences of HIV-2 and SIV isolates. The dendrogram was unrooted. The personal computer gene sequence analysis software (version 6.5) was used (program Clustal; Intelligenetics). The DNA of the isolate HIV-2_{ISY} was resequenced from the original molecular clone (11), and three nucleotide differences from the published sequence were found at positions 6910 (A instead of C), 7043 (G instead of A), and 7247 (G instead of A), reflecting previous sequencing errors.

2_{ISY}, HIV-2_{D194}, HIV-2_{NIH-Z}, and HIV-2_{GB52}. In this analysis, HIV-2_{ROD}, from the Cape Verde islands, appeared to be the most divergent.

In conclusion, the genetic variability among the HIV-2 isolates analyzed in this study appeared to be comparable to that among HIV-1 isolates. Recently, very divergent viruses have been identified among African HIV-1 isolates (32) and among West African HIV-2 isolates from a remote region in Liberia (15). Thus, determination of the extent of interspecies and intraspecies variability of these human lentiviruses awaits further studies.

In monkeys experimentally inoculated with a molecular clone of HIV-2 (13) and three independent molecular clones of SIV (4, 5, 23, 35), variability over time in the V3 region has been reported to be low. In those experiments, the analysis of the proviral DNA was performed at different lengths of time after infection, the longest being 2.5 years. More extensive observation over time as well as the analysis of a large number of animals might provide data that parallel what we observed with individuals naturally infected with HIV-2.

Epidemiological data indicate that HIV-2 is less pathogenic than HIV-1 (29) and that HIV-2 is not readily transmitted to offspring and infected patients (37). Thus, HIV-2 represents a lesser concern for public health. Clearly, it is a higher priority to develop a vaccine against HIV-1 than it is to develop one against HIV-2. However, since HIV-2 can be used in an animal model for vaccine development against HIV-1, the understanding of genetic, biological, and functional analogies between these two lentiviruses is important. Even more important is the understanding of the host immune response to these viral agents. Recently, it has been shown that immunization of rhesus macaques and rodents with *Salmonella typhimurium* carrying HIV-2 *gag* and *env* antigens elicited a cell-mediated immune response directed against HIV-2 epitopes, which map at positions equivalent to those found for HIV-1 (although the epitope sequences differed) (1). Here we show that another important region (V3) of the HIV-2 envelope varies in nature to a degree equivalent to HIV-1.

In summary, we believe that these data suggest that HIV-2 and its close relative SIV represent acceptable models to study protective immunity in vaccine trials before using scarce chimpanzees.

Part of this work was supported by ASCOM; GVC; ICSC World Laboratory, project MCD-2/11; and MedImmune (Gaithersburg, Md.).

We are grateful to R. C. Gallo for support and Linda Anderson for editorial assistance.

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