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Genetic variability between isolates of human immunodeficiency virus (HIV) type 2 is comparable to the variability among HIV type 1

(human acquired immunodeficiency syndrome/DNA sequence)

J. F. ZAGURY*, G. FRANCHINI*†, M. REITZ*, E. COLLALTI*, B. STARCICH*, L. HALL*, K. FARGNOLI*, L. JAGODZINSKI‡, H.-G. GUO*, F. LAURE§, S. K. ARYA*, S. JOSEPHS*, D. ZAGURY§, F. WONG-STAAL*, AND R. C. GALLO*

*Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; ‡Biotech Research, Inc., Rockville, MD 20852; and §Universitaire Pierre et Marie Curie, Paris, France

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ABSTRACT **The isolation from macaques of retroviruses related to human immunodeficiency virus (HIV) led to the identification of a second group of human retroviruses (termed HIV-2), which are prevalent in West Africa and closely related to the simian immunodeficiency virus (SIV). We have cloned and determined the complete nucleotide sequence of the human West African retrovirus HIV-2_{NIH-Z} and compared it to that of a previously described strain of HIV-2 (HIV-2_{ROD}) as well as to SIV and HIV-1. We have reached the following conclusions: (*i*) The HIV-2 isolates are (slightly) more closely related to each other than to SIV, compatible with their isolation from different species. (*ii*) The variability between HIV-2 isolates is similar in degree and kind to that found among HIV-1 isolates. The equivalent degrees of intragroup divergence suggest that HIV-1 and HIV-2 have existed in their present ranges in Africa for approximately equal lengths of time. The fact that acquired immunodeficiency syndrome is widespread in regions where HIV-1 is prevalent but not in regions where HIV-2 is prevalent suggests a substantial difference in the morbidity rates associated with HIV-1 vs. HIV-2 infection. (*iii*) HIV-2 and SIV are related to each other more closely than they are to HIV-1.**

Since 1981, when the early reports of cases of the acquired immunodeficiency syndrome (AIDS) were published (1), two groups of human retroviruses have been discovered and characterized in addition to the previously described human retroviruses, human T-cell lymphotropic virus 1 and 2 (HTLV-1 and HTLV-2) (2–4). The first, human immunodeficiency virus (HIV)-1 (5–7), is prevalent in AIDS cases worldwide and seems to be the primary cause of the severe T-cell depletion observed in patients with AIDS. The existence of the second group of human retroviruses was shown (8) by a serological study of a healthy West African population, using as target antigens the proteins of a retrovirus [simian immunodeficiency virus (SIV)] discovered in macaques (9). SIV was identified by the serological cross-reactivity of its major core protein with that of HIV-1 (10). Simultaneously, the identification of West African patients with frank AIDS, who were apparently not infected with HIV-1, led to the isolation of human retroviruses (HIV-2) distinct from HIV-1 but related to SIV (11, 12). A third group (13) reported the isolation from a West African patient of another retrovirus, closely related to SIV and distantly related to HIV-1, which they designated HIV-$2_{SBL6669}$.

We report here the complete nucleotide sequence¶ of an HIV-2 isolate from a patient with immunodeficiency and compare it with sequences from the previously described HIV-2_{ROD} (11, 12), HIV-1, and SIV.

MATERIALS AND METHODS

Virus Isolation and Cloning of Proviral DNA. Fresh peripheral blood cells from an infected individual were cocultivated with phytohemagglutinin-stimulated peripheral mononucleated cells from a healthy donor by following previous procedures (2). Viral particles and reverse transcriptase were identified after a few weeks. The virus, designated HIV-2_{NIH-Z}, was transmitted to the human CD4-antigen-positive neoplastic T-cell line HUT-78 by cocultivation of the infected peripheral mononucleated cells (6).

High molecular weight DNA from the HIV-2_{NIH-Z}-infected cell line HUT-78 was partially cleaved with *Bam*HI restriction endonuclease and selected by size on a sucrose gradient. The DNA fraction containing 20-kilobase (kb) fragments was purified by precipitation with ethanol. The DNA was ligated into the *Bam*HI site of EMBL3 and the recombinant phages were plated and then screened with plasmids SS35 and B16, which are probes containing *gag* and *env* regions, respectively, of SIV (14).

DNA Sequencing. Fragments of proviral DNA from the phage library were subcloned in Bluescribe (Stratagene, San Diego, CA) or M13mp8 and M13mp9. The DNA sequence was obtained by the dideoxy chain terminator procedure (15), using synthetic primers and either the Klenow fragment of *Escherichia coli* DNA polymerase or T7 DNA polymerase (Sequenase, United States Biochemical, Cleveland, OH). Some (3 kb) of the proviral DNA was also sequenced by the method of Maxam and Gilbert (16).

RESULTS

Nucleotide Sequence and Genomic Organization of the HIV-2_{NIH-Z} Provirus. We isolated HIV-2_{NIH-Z} in 1986 from the peripheral blood of an immunodeficiency patient who originally lived in Guinea Bissau. A virus, isolated from the same patient and designated lymphadenopathy–AIDS virus (LAV)-2_{FG}, was described by Clavel *et al.* (11) and is likely highly similar. HIV-2_{NIH-Z} was propagated first in human peripheral blood cells and later in the human neoplastic cell

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Abbreviations: AIDS, acquired immunodeficiency syndrome; HTLV, human T-cell lymphotropic virus; HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; LTR, long terminal repeat; ECR, envelope conserved region.

†To whom reprint requests should be addressed.

¶The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03654).

line HUT-78. Using SIV proviral DNA as a probe (14), we isolated molecular clones containing complete and incomplete proviral copies and determined their nucleotide sequence. The complete sequence has been deposited in the EMBL/GenBank data base.¶ The HIV-2_{NIH-Z} genome is 9431 base pairs long and the organization of the open reading frames is consistent with the order 5'LTR–*gag*–*pol*–middle region–*env*–3'*orf*–3'LTR (LTR, long terminal repeat), similar to HIV-1 and SIV (17–23). A single difference lies in the presence in HIV-2_{NIH-Z} of an extra open reading frame in the middle of the genome which is absent from the HIV-1 genome but present in HIV-2_{ROD}, SIV, and SIV_{mac}, called *X* (21, 22, 24).

The LTR. The HIV-2_{NIH-Z} LTR is 632 nucleotides. The sizes of the U3, R, and U5 regions were derived by analogy with HIV-2_{ROD} and SIV and are 329, 176, and 127 nucleotides, respectively. While the U5 and R are comparable in size to those of HIV-2_{ROD} and SIV, the U3 of the HIV-2_{NIH-Z} is shorter by 228 nucleotides, due to a deletion in the HIV-2_{NIH-Z} U3 region 60 nucleotides 3' of the polypurine tract. Since the deletion is present in both the 5' and 3' LTRs, it is probably present in the provirus (and is not a cloning artifact). The positions of the canonical regulatory sequences in the HIV-2_{NIH-Z} LTR, such as the TATAA box, the polyadenylylation signal AATAAA, and the transcription factor SP1 binding sites, have been identified.

The *gag* and *pol* Gene Proteins. The first open reading frame corresponds to the gag precursor. In infected cells the size of the gag precursor appears to be 55 kDa as judged by immunoprecipitation with human sera from infected individuals (13). The *gag* open reading frame has a coding capacity of 519 amino acids, consistent with p55 being the gag precursor, as with HIV-1 (17–20). We performed protein alignments by using the algorithm of Dayhoff and colleagues (25). The amino acid identities between the gag precursor polypeptide of HIV-2_{NIH-Z} and the polypeptides of HIV-2_{ROD}, SIV, and the HTLV-IIIB strain of HIV-1 are 92%, 82%, and 52%, respectively (Table 1), indicating that the two human HIV-2s are more closely related to each other than to SIV and that HIV-1 is more distantly related to all of them. The cleavage site for the major core protein (p24) in the HIV-2_{NIH-Z} gag precursor was provisionally assigned by alignment of the amino acid sequences of the gag precursor polypeptides of HIV-2, HIV-1, and SIV.

The *pol* open reading frame encoding 1190 amino acids overlaps the *gag* precursor open reading frame, similar to HIV-1. The overall amino acid sequence relationships among the *pol* genes of both HIV-2 isolates, HIV-1, and SIV are like those of the *gag* genes and lead to the same conclusions.

Conserved and Variable Domains in the Envelope Proteins of HIV-1, HIV-2, and SIV. The third major open reading frame in the HIV-2_{NIH-Z} provirus corresponds to the envelope protein (Fig. 1) and potentially encodes 856 amino acids. A comparative analysis of the envelope protein of HIV-2_{NIH-Z} with HIV-2_{ROD} and SIV showed an overall identity of 80% and 70%, respectively. The degree of conservation is comparable in the extracellular and transmembrane portions of the envelope proteins (Table 1). The envelope proteins of HIV-2_{NIH-Z} and HIV-1 are much less related (35%), as shown in Table 1. This comparison, however, identifies regions that are relatively conserved and thus likely to be crucial for envelope protein function. The positions of the cysteines are highly conserved among all these retroviruses; in the extracellular envelope protein 22 cysteine residues are conserved among both HIV-2 isolates and SIV (Fig. 1), and 19 of 22 are also present in the same position in all strains reported of HIV-1 (data not shown). Similarly, in the transmembrane portion of the envelope protein 3 cysteines are conserved among both strains of HIV-2 and SIV and 2 are also conserved in the HTLV-IIIB strain of HIV-1. Clearly, disulfide bonds must play a crucial role in maintaining the secondary structure of the envelope proteins.

Further analyses of the amino acid homology among the envelope proteins identified regions in which either complete amino acid identity or only conservative changes could be detected in all these viruses. Of these ECRs, indicated in Fig. 1, ECR-6, located in the extracellular glycoprotein, is a putative binding site for the CD4 molecule (26), an essential part of the cellular receptor for HIV-1, SIV (27–29), and very likely HIV-2. A peptide containing the ECR-6 sequence from HIV-1 has been shown to induce cell-mediated immunity in mice (30). Recently it has been hypothesized that the first 11 amino acids of ECR-7 may be [based on the homology of this region with the fusion peptide of human paramyxoviruses, measles, and respiratory syncytial viruses (31)] the fusion peptide of HIV-2, HIV-1, and SIV. The 3'-most conserved region in the transmembrane envelope protein (ECR-12) has been implicated in the viral cytopathic effect *in vitro* (32), although this has been disputed (33). In any case, conservation of these 17 amino acids in HIV-1, HIV-2, and SIV suggests that this region is important.

A 33-kDa protein in HIV-2_{NIH-Z}-infected cells (compared to 41 kDa protein in HIV-1-infected cells) is thought to be the transmembrane envelope protein (unpublished data). A similarly truncated form of the transmembrane protein has been identified in SIV-infected cells (8). The *env* gene sequence of SIV contains a termination codon that would eliminate the carboxyl-terminal 146 amino acids of the transmembrane protein (21–23). In contrast, our clone from HIV-2_{NIH-Z}-infected cells does not have a termination codon at this position. Since the cells from which it was isolated appear to express a truncated gp33, the provirus that we cloned is likely not representative of the majority of the proviruses present. Although the significance of the stop codon in some of these viruses is not clear, it might be important in helping to modulate their biological properties. A stop codon is present in precisely the same position in some clones of HIV-2_{ROD} (22). Furthermore, the identity between SIV and HIV-2 transmembrane envelope proteins decreases dramatically from 82% before the stop codon to 55% after it (Fig. 1). Taken together, these data suggest that this stop codon has biological significance.

The hypervariable regions identified for HIV-1 isolates (34) are also generally the most variable in both HIV-2 isolates and SIV (Fig. 1). The first region of variability among the envelope proteins of HIV-2_{NIH-Z}, HIV-2_{ROD}, and SIV spans from amino acid residue 112 to residue 190 in HIV-2_{NIH-Z} and corresponds to the hypervariable region from residue 130 to residue 210 in HIV-1 (34, 35). We analyzed the degree of variability in this region among HIV-2 and SIV isolates and among HIV-1 isolates and found that the percentage of amino

Table 1. Percent amino acid identity of viral proteins from viral isolates compared with HIV-2_{NIH-Z} proteins

Isolate	gag	pol	env			sor	tat	trs/art	X	R
			Total	ECP	TMP					
HIV-2_{ROD}	92	91	80	78	84	86	85	86	78	69
SIV	82	75	70	68	72	64	63	59	—	—
HIV-1 (HTLV-IIIB)	52	54	35	32	39	28	29	34	—	37

ECP, extracellular portion; TMP, transmembrane portion.

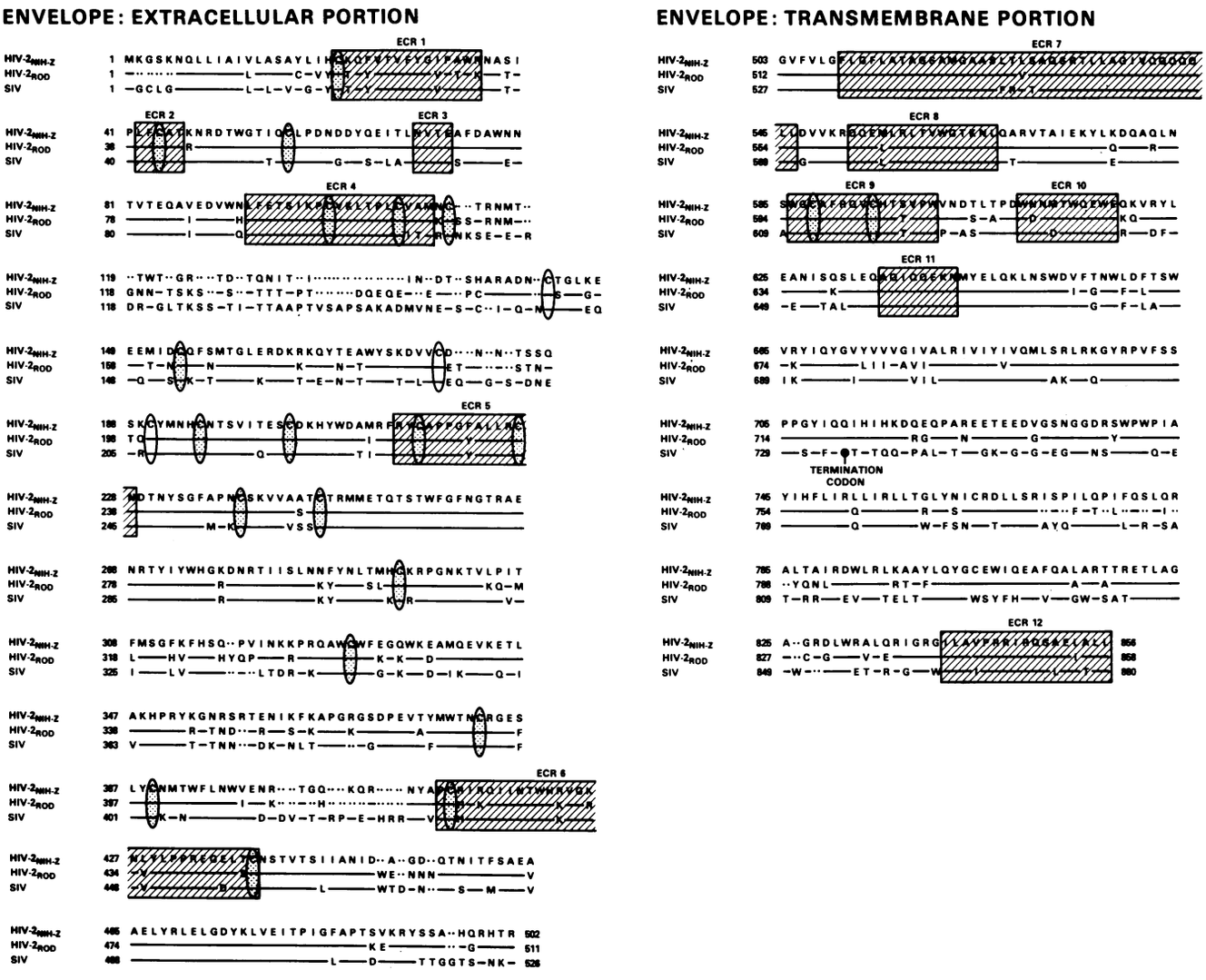

FIG. 1. Identification of variable and conserved regions in the envelope proteins of HIV-1 and HIV-2. The amino acid alignment of the envelope proteins of HIV-2_{NIH-Z}, HIV-2_{ROD}, and SIV is shown. The left part of the figure represents the extracellular envelope proteins and the right part contains the transmembrane portion. The amino acid sequence (standard one-letter symbols) of the envelope of HIV-2_{NIH-Z} is the upper line of each row. A continuous line represents amino acid identity and a dotted line stands for the lack of the amino acid. The hatched boxes include regions [envelope conserved regions (ECRs)] that have relatively high amino acid identity with the HTLV-IIIB strain of HIV-1. The stippled ovals include the cysteine residues that are conserved in the same position in both HIV-2 isolates, SIV, and HIV-1 (HTLV-IIIB). The empty ovals represent the cysteine residues that are conserved only in the West African viral isolates and SIV. The location of the termination codon in the SIV transmembrane envelope protein is indicated.

acid identity is 40–50% in the former group (Table 2) and 30–60% in the latter group (see Table 3). This may mean that both groups of viruses may have spread to their present ranges from a limited focus of infection at approximately the same time.

Identification of Putative Functional Domains of Other Viral Proteins. Several other genes have been identified in the HIV-1 genome by immunological (36–40) or functional (41–43) studies. The corresponding genes can be identified in the HIV-2 genome and the comparative analyses of their amino acid sequence identify strongly conserved domains within some of them.

The HIV-2 *trs/art* gene, which was discovered in HIV-1 by mutagenesis of a biologically active HIV-1 clone (41–43), seems to be crucial for the expression of the HIV-1 envelope protein (41). Protein sequence alignments of the HIV-2 and HIV-1 *trs/art* gene products show an arginine-rich region in the second coding exon that is conserved among HIV-1, HIV-2, and SIV (Fig. 2). Similarly, arginine- and cysteine-

Table 2. Comparison of a variable region in HIV-2 and SIV envelope proteins

	% amino acid identity		
	HIV-2_{NIH-Z}	HIV-2_{ROD}	SIV
HIV-2_{NIH-Z}	100	—	—
HIV-2_{ROD}	52	100	—
SIV	40	41	100

The residues compared are numbered 112–190 in the HIV-2_{NIH-Z} protein, 109–199 in HIV-2_{ROD}, and 121–217 in SIV.

Table 3. Comparison of a variable region, amino acid residues 130–210, in HIV-1 envelope proteins of various isolates

	% amino acid identity				
	HTLV-IIIB	ELI	ARV-2	MAL	HTLV-IIIRF
HTLV-IIIB	100	—	—	—	—
ELI	52	100	—	—	—
ARV-2	60	55	100	—	—
MAL	48	52	55	100	—
HTLV-IIIRF	43	37	40	31	100

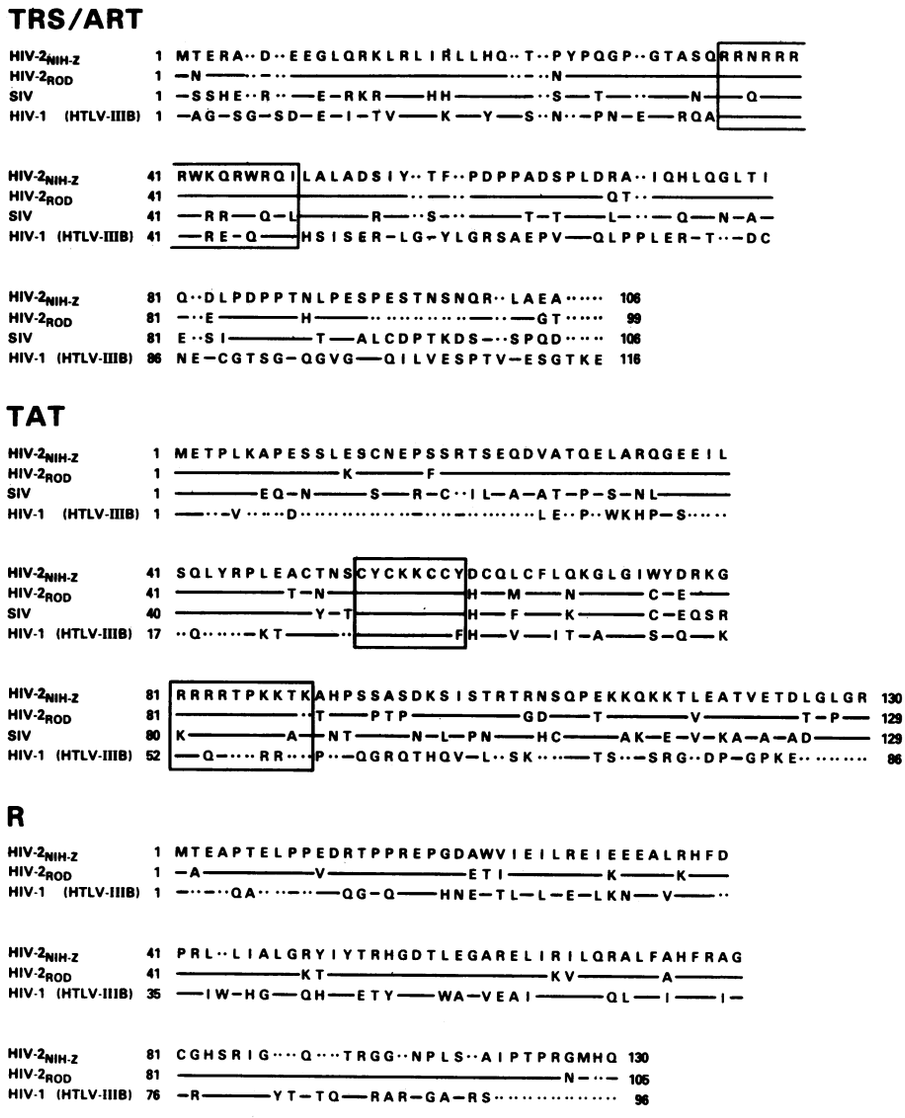

FIG. 2. Amino acid alignment of other viral proteins. The amino acid sequence of the HIV-2_{NIH-Z} trs/art, tat, and R presumed proteins is represented in the top line of each alignment. The continuous line represents amino acid identity among the viral isolates. The boxes include regions that are also conserved in HIV-1 (HTLV-IIIB).

rich regions (Fig. 2) can be identified in the first coding exon of the tat proteins, which are responsible for the transactivation of virus expression in these viral isolates (39, 41, 43). No recognizable conserved regions were detected within the *sor* (*Q*) gene, although the sor proteins of these viruses have similar hydropathy profiles (not shown). A highly conserved region could, however, be identified in the 3'*orf* (*F*) gene, although a correct protein alignment of the 3'*orf* protein product could not be made because of the presence of the 228-nucleotide deletion in U3.

DISCUSSION

The discovery of a second group of viruses in both primates and humans that are structurally and genetically related to HIV-1 and are also associated with immunodeficiency may call for a reevaluation of the hypothesis that AIDS is a new disease. Documented cases of AIDS-like diseases or aggressive forms of Kaposi sarcoma in young people date as far back as the early 1970s (44–46). One point of considerable debate is to what extent the West Africa viruses cause AIDS in people. Viruses of the HIV-2 subgroup were discovered by Kanki *et al.* (8) by a serological study on healthy people from Senegal. Early reports, which identified a human virus related to SIV, on the basis of serology, suggested a lack of disease association (8), while others reported the isolation of HIV-2 viruses from a few patients with immunodeficiency and no signs of infection with HIV-1 (11). More recently, an increasing number of HIV-2 isolates have been obtained from patients with AIDS from West Africa (47). However, a retrospective seroepidemiological study on 4248 people in West Africa showed the absence of any clinical signs in 330 infected people (48). The *env* gene variance of two HIV-2 isolates is similar in kind and degree to that of HIV-1, which suggests that HIV-1 and HIV-2 have existed in their present populations for similar lengths of time. It is, therefore, possible that the discrepancy between HIV-2 studies is due to differences in the populations examined and that HIV-2 infection has a lower morbidity rate. A more difficult question is whether there is a fundamental genetic difference between the two virus groups that could explain such a difference in infected individuals. The overall genetic structures of HIV-1 and HIV-2 are very similar, with the exception of an extra open reading frame in HIV-2, which has been designated *X*. *X* is translated *in vivo* and encodes a protein of 16 kDa in HIV-2-infected cells (49), but its function is unknown. The presence of a stop codon in the transmembrane portion of the *env* gene of some of these viruses opens

the possibility that two different forms of this protein could be synthesized. The biological significance of these genetic differences between HIV-1 and HIV-2 remains to be investigated.

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