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Biological and Molecular Variability of Human Immunodeficiency Virus Type 2 Isolates from The Gambia

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Seven new human immunodeficiency virus type 2 (HIV-2) isolates (CBL-20 to CBL-26) from The Gambia were characterized. Their cytopathogenicity and growth in vitro correlated with the severity of clinical disease. CBL-22 was highly sensitive to neutralization by HIV-2 sera and was cross-neutralized by some HIV-1 sera. These findings, the differing sizes of envelope glycoproteins of individual isolates, and the sequence analysis of amplified regions of the viral DNAs show that these HIV-2 isolates from one geographical region in West Africa exhibit biological and genome variability comparable to that observed for HIV-1.

Human immunodeficiency virus type 2 (HIV-2) is endemic in regions of West Africa (11, 12, 32) and has been observed occasionally in Europe (5, 24) and North America (7). HIV-2 is pathogenic in humans, causing an immunodeficiency disease indistinguishable from that associated with HIV-1 (6, 11, 12, 15, 24). However, epidemiological evidence suggests that some HIV-2-positive asymptomatic individuals may progress more slowly towards clinical disease than would be expected for HIV-1 infection (26; D. Ricard, S. M'Boup, A. Ndoye, P. J. Kanki, F. Denis, and A. Gueye, Abstr. 3rd Int. Conf. AIDS Associated Cancers Africa, 1988, p. 102). A seroprevalence study in Guinea-Bissau, West Africa, showed that, in contrast to HIV-1 in Central Africa, the prevalence of HIV-2 was the same in older individuals as in the most sexually active age groups (30). These studies, as well as case reports (14), suggest that HIV-2, or at least some HIV-2 isolates, may have a longer incubation period than is usually seen for HIV-1. Because of the similarities between HIV-2 and the two simian immunodeficiency viruses SIV_{mac} (8) and SIV_{sm} (20), HIV-2 may be derived from the latter or a closely related simian lentivirus (27). It was therefore of interest to investigate the spectrum of HIV-2 isolates existing in a given geographic region of West Africa with respect to their biological properties and molecular variability.

We obtained blood from 20 seropositive individuals attending the hospital or the clinic for sexually transmitted diseases in Fajara, The Gambia, after informed consent had been given. The serological diagnosis of HIV-2 infection was made on the basis of negative HIV-1 and positive HIV-2 competitive enzyme-linked immunosorbent assays (36) and Western blot (immunoblot) analysis. Mononuclear cells were cultured in RPMI 1640 medium with phytohemagglutinin and, after 3 days, interleukin-2. Uninfected cord blood mononuclear cells were added after 7 days. Seven syncytium-producing HIV-2 isolates derived from different disease categories were obtained (Table 1). The individuals from whom CBL-23, -24, -25, and -26 were isolated had worked as prostitutes. No information was available on the sexual contacts of the others. All seven individuals were from an urban environment.

The seven isolates differed in their growth rates, cytopathogenicity in vitro, and sensitivity to neutralizing antibodies in patient sera. CBL-20 and CBL-21 grew rapidly in primary culture and produced high levels of reverse transcriptase, measured as described previously (22), within 14 days (Table 1). In contrast, CBL-22 and CBL-23 took somewhat longer to produce significant reverse transcriptase activity and CBL-25 and CBL-26 gave rise to only low reverse transcriptase levels after 4 weeks. Whereas CBL-20, -21, -22, and -23 grew in H9, MOLT4, C8166, CEM, and U937 cells (10, 25), CBL-24 grew only in the MOLT4 cell line after several attempts. CBL-25 and CBL-26 could not be established in any of these permanent cell lines.

Table 1 also shows that there was a close correlation between in vitro cytopathogenicity of the different isolates and the clinical disease status of the individuals from whom they were obtained. Samples from the two patients with acquired immunodeficiency syndrome (AIDS) gave rise to CBL-20 and CBL-21, the two most highly cytopathogenic strains. CBL-22 and CBL-23, which took slightly longer to appear in primary culture, were from patients who died in the meantime or whose condition progressed to AIDS or AIDS-related complex, whereas CBL-24, -25, and -26 were from asymptomatic individuals. The two subjects from whom CBL-25 and CBL-26 were isolated have remained asymptomatic for 3 years. For HIV-1 isolates, such a relationship has been previously described (3, 37). Our findings as well as a recent study (1) would therefore suggest that a similar relationship between in vitro cytopathogenicity and clinical disease exists for HIV-2.

We examined the sensitivity of CBL-20, -21, -22, and -23 to neutralization by human sera from the same region of West Africa. Neutralization assays (28, 39) were performed with 24 HIV-2 sera, 7 HIV-1 sera, and 7 sera which were dually reactive by competitive enzyme-linked immunosorbent assays. Geometric means and range of neutralization titers (serum dilutions inhibiting >98% of syncytia) are shown in Table 2. CBL-20, -21, and -23 behaved similarly to most HIV-1 strains and previously published HIV-2 isolates (39) by being neutralized only by low dilutions of most sera. In particular, they were not neutralized by HIV-1 antisera, confirming a previous report for lymphadenopathy virus type 2 (LAV-2) ROD and SBL 6669 (39). In contrast, CBL-22 proved to be extremely sensitive to neutralization by all HIV-2-positive sera and also by some HIV-1-positive

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TABLE 1. Biological characteristics of HIV-2 isolates[a]

Isolate	Sex	Age (yr)	Clinical status	T cells/μl		Disease progression	Reverse transcriptase activity in cord MNC	CPE and replication in:		
				CD4	CD8			Cord MNC	MOLT4 clone 8	C8166, H9, CEM, U937
CBL-20	M	28	AIDS, diarrhea, wasting, oral thrush	18	133	Dead	+ (5 days, 6,000 cpm [7× background]), +++ (21 days, 80,000 cpm [50× background])	+	+	+
CBL-21	F	34	AIDS, diarrhea, wasting	25	563	AIDS	+ (14 days, 4,000 cpm [8× background])	+	+	+
CBL-22	M	55	ARC, diarrhea, wasting, oral thrush	Total 680		Dead	+ (21 days, 8,000 cpm [16× background])	+	+	+
CBL-23	F	31	ASY	ND	ND	ARC	+ (21 days, 8,000 cpm [9× background])	+	+	+
CBL-24	F	22	ASY	ND	ND	PGL	+ (28 days, 6,000 cpm [15× background])	+	+	−
CBL-25	F	45	ASY	1,660	1,010	ASY	+ (28 days, 1,500 cpm [3× background])	+	−	−
CBL-26	F	40	ASY	ND	ND	ASY	+ (28 days, 1,500 cpm [3× background])	+	−	−

[a] Clinical and laboratory data of the individuals from whom the HIV-2 CBL isolates were obtained were compared with biological characteristics of these isolates. For CBL-22, no values for CD4 and CD8-positive T cells were available and therefore the total lymphocyte count is given. MNC, Peripheral blood mononuclear cells; CPE, cytopathic effect; PGL, persistent generalized lymphadenopathy; ARC, AIDS-related complex; ASY, asymptomatic subject; cpm, [^{3}H]thymidine incorporation; ND, not done.

TABLE 2. Neutralization sensitivity of HIV-2 isolates

Serological classification (n)[a]	Isolate neutralization titers[b]											
	CBL-20		CBL-21		CBL-22		CBL-23		LAV-2		HIV-1 RF	
	GM	R	GM	R	GM	R	GM	R	GM	R	GM	R
HIV-2, neutralizing HIV-2 only (16)	10.5 (1.02 ± 0.45)	<10–80	6.5 (0.8 ± 0.31)	<10–40	1,452.1 (3.2 ± 0.72)	40–6,250	10.5 (1.02 ± 0.37)	<10–40	28.2 (1.45 ± 0.77)	<10–640	<10	
HIV-2, neutralizing HIV-2 and HIV-1 (8)	9.3 (0.97 ± 0.39)	<10–50	6.6 (0.82 ± 0.35)	<10–50	2,006.8 (3.3 ± 0.73)	160–31,250	17.38 (1.24 ± 0.49)	<10–80	66.1 (1.82 ± 0.57)	<10–640	36.2 (1.56 ± 0.397)	20–320
HIV-1 (7)	6.9 (0.84 ± 0.26)	<10–50	<10		46.8 (1.67 ± 0.72)	20–1,250	<10		6.02 (0.78 ± 0.23)	<10–20	42.38 (1.63 ± 0.472)	40–250
HIV-1 and HIV-2 (7)	18.03 (1.26 ± 0.70)	<10–160	12.02 (1.08 ± 0.38)	<10–40	4,223.9 (3.63 ± 0.715)	1,250–156,250	8.18 (0.91 ± 0.29)	<10–50	120.2 (2.08 ± 0.81)	<20–640	19.49 (1.29 ± 0.30)	<10–40

[a] n, Number of sera tested.

[b] CBL isolates, an HIV-2 reference strain (LAV-2 ROD), and an HIV-1 reference strain (human T-cell lymphotropic virus type III RF) were tested. Sera were classified as HIV-2 positive, HIV-1 positive, or dually reactive in ELISA (see text), and neutralization tests were performed as described previously (28, 39). The geometric mean (GM) of neutralization titers, with the logarithm of the geometric mean and its standard error in parentheses, is given. For the calculation of the geometric mean, neutralization titers of <1:10 were treated as 1:5. If all sera in one category were negative in neutralization, no geometric mean was calculated and the result is represented as <10. The range (R) of neutralization titers is also given.

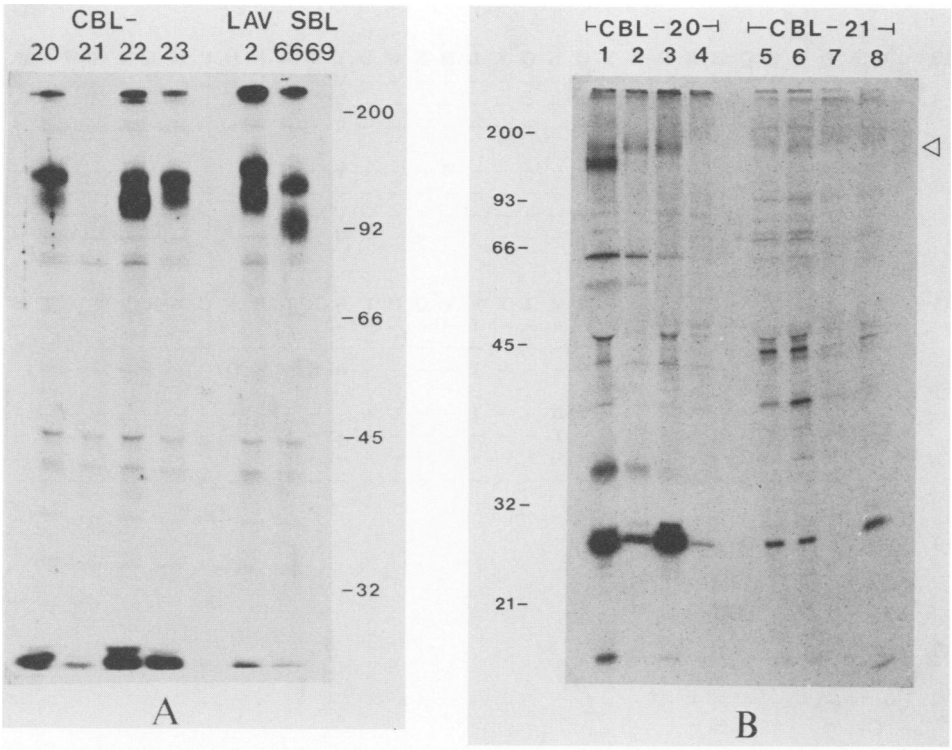

FIG. 1. Radioimmunoprecipitation assay of HIV-2 strains. Four HIV-2 CBL isolates and two HIV-2 reference strains (ROD and SBL-6669) were used to infect H9 cells. Infected cells (A) and cell-free supernatant (B) were immunoprecipitated with a pool of 15 human HIV-2-positive sera derived from Senegal, Guinea-Bissau, and the Ivory Coast (all lanes in panel A; lanes 1 and 5 in panel B), a pool of HIV-2 sera with neutralizing activity against CBL-21 (panel B, lane 6), plasma from a cynomolgus macaque infected with SIV_{mac} (panel B, lanes 2 and 7), plasma from a cynomolgus macaque infected with SIV_{sm} (panel B, lanes 3 and 8), and normal human serum (panel B, lane 4). The arrowhead indicates the position of the envelope precursor protein of CBL-21. Numbers in the margins show molecular sizes in kilodaltons.

sera. This isolate can thus be used to identify neutralizing antibodies in sera which neutralize other HIV-2 strains only marginally or not at all and to analyze cross-neutralizing antibodies in HIV-1 sera. Although reactivity of some HIV-1 sera with the envelope of HIV-2 has been reported previously by using Western blots (36) or recombinant envelope antigens derived from LAV-2 ROD (35), only moderate sensitivity to cross-neutralizing HIV-1 antisera has been reported for the SBL K135 isolate of HIV-2 (4).

In being unusually sensitive to neutralization, the CBL-22 isolate resembles the SF-2 isolate of HIV-1 (9, 40). These isolates may therefore be useful to study the epitopes responsible for cross-reactive neutralizing antibodies. It is unclear why some HIV isolates should be so much more sensitive than others, though this may relate to differences in antigenic epitope expression or to the number and presentation of envelope glycoprotein molecules on an infectious virion.

To analyze the envelope glycoproteins of these isolates, they were immunoprecipitated from infected H9 cells or cell culture supernatant which had been previously labeled with 50 μCi of [^{35}S]cysteine and of [^{35}S]methionine (Amersham Corp.) per ml at a concentration of 10^7 cells per ml.

Figure 1 shows that the molecular weight of the envelope precursor and the outer envelope protein of different HIV-2 isolates are variable and that all the CBL isolates as well as SBL-6669 (2) have smaller envelope proteins than the prototype strain, LAV-2 ROD. These differences cannot be wholly due to truncated transmembrane proteins, as described for a subclone of HIV-2 ST (23) and SIV_{mac} (19), since the outer envelope proteins also differ in size. In addition, no stop codons were detected in the transmembrane region of CBL-20, -21, -22, and -23 (Fig. 2), which contains these stop codons in SIV_{mac} (19), although we cannot exclude stop codons in other parts of the envelope. A similar observation has recently been reported for the HIV-2 EHO isolate (31), whose smaller envelope size was due to a shorter protein core. For CBL-23, polymerase chain reaction (PCR) amplification of the complete *env* gene with primers anchored in *tat* (position 5834 of HIV-2 ROD [17]) and downstream of *env* (position 8885) resulted in a band of approximately 2.8 kilobases, whereas the corresponding PCR product obtained with HIV-2 ROD had a size of 3.0 kilobases (data not shown). This suggests that for CBL-23 at least, a shorter protein sequence is responsible for the smaller envelope protein. Whereas the envelope proteins of CBL-20, -22, and -23 reacted well in immunoprecipitation assays with a pool of randomly selected HIV-2-positive human sera, no, or only a weak, envelope band could be immunoprecipitated from CBL-21 in several experiments, even when a pool of six human sera selected for their capacity to neutralize CBL-21 at titers of 1:20 and 1:40 (Fig. 1B, lane 6) or plasma from SIV_{mac}- and SIV_{sm}-infected animals (lanes 7 and 8) was used. This probably reflects a lower level of viral protein production in CBL-21 cultures and indicates that CBL-21 does not fall into the same category as HIV-2 D205, a divergent HIV-2 isolate recently reported to be equidistant from HIV-2 ROD and SIV_{mac} or SIV_{sm} (13).

An approximately 5-kilobase *Bam*HI restriction fragment

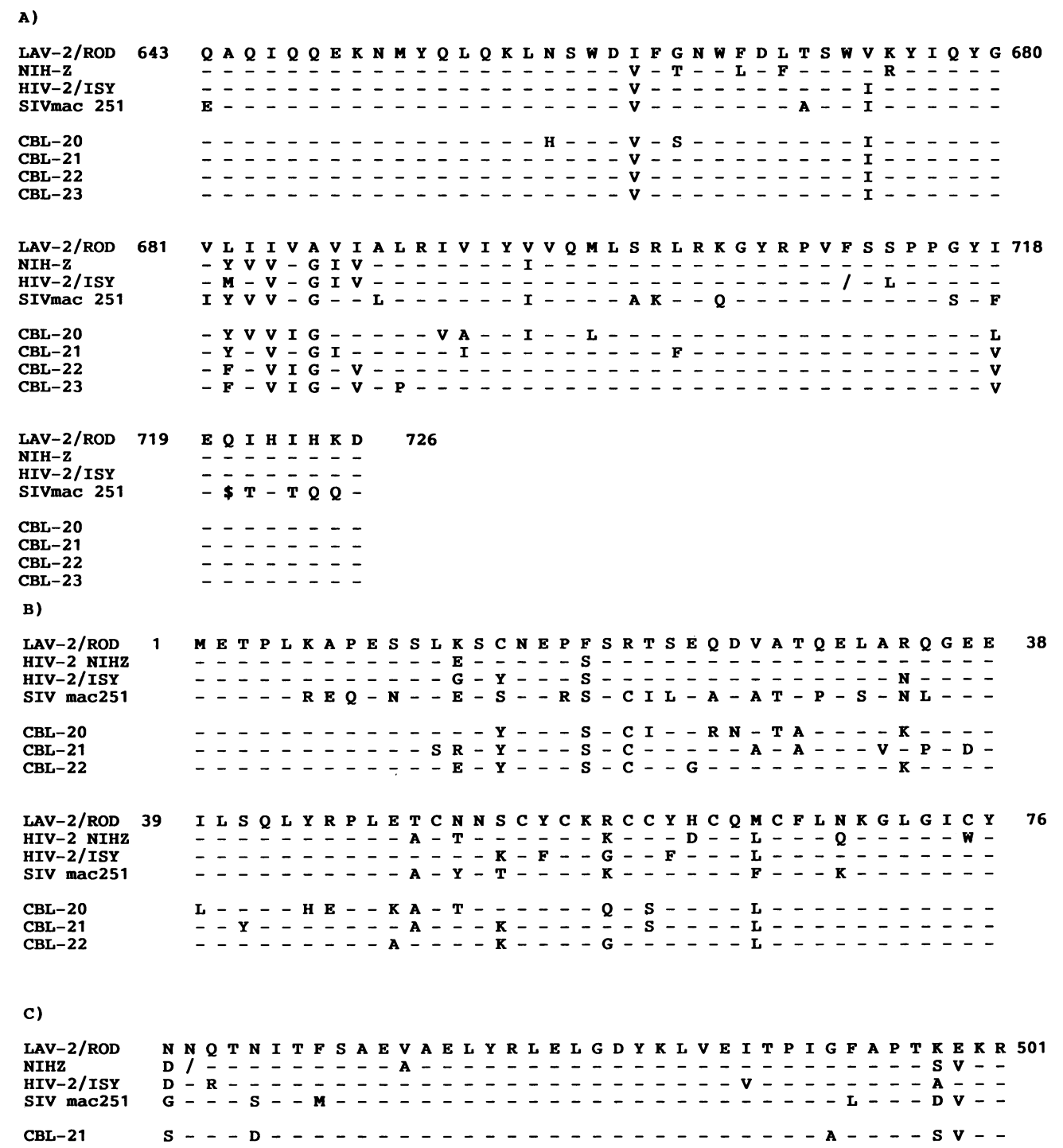

FIG. 2. Amino acid sequences derived from amplified genomic regions of CBL-20 through -23. Cloned PCR fragments of CBL-20 through -23 were sequenced to derive amino acid sequences for a region in the transmembrane protein of *env* (A) and the first *tat* exon (B). The C-terminal 40 amino acids of the outer envelope glycoprotein (gp120) of CBL-21 (C) were derived from a directly sequenced PCR fragment. Alignment with published HIV-2 and SIV sequences is shown. $, Stop codon present in the simian immunodeficiency virus envelope. Numbering refers to amino acids of *env* (A and C) or *tat* (B) according to the HIV-2 ROD sequence (17).

derived from LAV-2 ROD (17), extending from *pol* into *env*, hybridized under conditions of high stringency to Hirt DNA (21) from C8166 cells infected with each CBL isolate, and each isolate could be distinguished by using one of the restriction enzymes *Bam*HI, *Pst*I, and *Eco*RI (data not shown).

For a more detailed analysis of the variability of these four isolates with respect to previously reported HIV-2 strains,

we used the PCR technique to amplify and sequence three short regions of their genomes. PCR was carried out as previously described (33) on Hirt DNA (21). For amplification of the first *tat* exon primers CTA TAC TAG ACA TGG AGA CA (positions 5834 to 5853 of LAV-2 ROD [17]) and CCT TTC GTT CAT AAC ATA TC (positions 6082 to 6063) and for the amplification of a fragment from the transmembrane protein (*env* amino acids 643 to 726), primers GAA CAG GCA CAA ATT CAG CAA (positions 8070 to 8090) and TCC CCG GTC CTT GTG GAT ATG (positions 8330 to 8310) were used. These regions were amplified from all isolates, and their sequences were determined (34) after cloning into the *Sma*I site of pUC19. Their amino acid sequence is shown in Fig. 2.

In contrast, another region containing the C-terminal 40 amino acids of the outer envelope of LAV-2 ROD, which has been shown for HIV-1 to contain an antibody epitope (29), could only be amplified from CBL-21 by using primers GGA GAA ATG TAT ATT TGC CTC (positions 7441 to 7461) and CAC ACC TCT TGT ATG TCT CC (positions 7685 to 7666) and was sequenced directly (41) with one of the primers used to generate the PCR fragment (CAC ACC TCT TGT ATG TCT CC). We estimate the error rate of Taq polymerase to be about 0.3×10^{-4} per cycle per base pair under these conditions on the basis of sequencing multiple independent clones derived from single PCRs, which is comparable to error rates observed by others (16). The most common errors observed were G-to-A and A-to-G transitions. Although a small number of sequence errors may therefore have been introduced into the sequences reported here, these did not affect the results of this study.

It is apparent that the sequences of CBL-20, -21, -22, and -23 are related to, but not identical with, those of LAV-2 ROD (17), NIH-Z (42), and SBL-6669 (HIV-2 ISY) (15). A valine-to-isoleucine change at amino acid position 674 of the envelope was found in all CBL isolates as well as in SBL-6669, another isolate from The Gambia. In the first exon of *tat*, the cysteine residue at position 15 was shifted six amino acids further downstream in all CBL isolates to a position where it was also found in $SIV_{mac}251$ (Fig. 2). As this cysteine residue does not exist in $SIV_{mac}142$ and as it is not part of the repeated cysteine motif starting at position 50 (8, 17), this change may not be of functional significance. The observed change from K-E-K-R (LAV-2 ROD) to S-V-K-R (CBL-21) in the carboxy-terminal region of the outer envelope protein could conceivably alter the epitope specificity. Whether this sequence is in fact part of an antibody epitope in HIV-2 and whether the observed changes alter its specificity will have to be examined by using synthetic peptides derived from this region. On a few occasions, several independent (up to three) clones from one PCR were sequenced. While this is not sufficient to draw conclusions similar to those for HIV-1 (16), they indicate that there is some limited intraisolate variability (about 2 to 3% on the nucleotide level), which is much less than the interisolate variability observed among CBL-20, -21, -22, and -23. In summary, a detailed characterization of four of seven HIV-2 isolates from The Gambia provides an example of the biological, antigenic, and molecular variability of HIV-2 infection in this small region of West Africa and extends the reports on genome variability of HIV-2 from other parts of Africa (11, 15, 18, 23, 24, 38, 42).

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