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Cytoplasmic Domain Truncation Enhances Fusion Activity by the Exterior Glycoprotein Complex of Human Immunodeficiency Virus Type 2 in Selected Cell Types

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To investigate the glycoprotein determinants of viral cytopathology, we constructed chimeric *env* genes between a noncytopathic strain of human immunodeficiency virus type 2 (HIV-2), designated HIV-2/ST, and a highly fusogenic and cytopathic variant derived from this virus. Expression of the resulting chimeric glycoproteins indicated that efficient syncytium formation in the human T-cell line Sup T1 mapped to the C-terminal region of the transmembrane (TM) glycoprotein subunit. In this region, the wild-type and cytopathic ST glycoproteins differed by only four amino acids and by the presence of a premature termination codon in the cytopathic variant. Subsequent site-directed mutagenesis indicated that the cytoplasmic domain truncation was responsible for the enhanced fusion activity. This modification, however, increased the fusion activity of the glycoprotein only in Sup T1 cells (in which the ST variant arose) but not in Molt 4 clone 8 or peripheral blood mononuclear cells. These observations indicate that the length of the cytoplasmic domain of the HIV-2 glycoprotein modulates the fusion activity of the exterior glycoprotein complex in a cell-specific manner. Such adaptability appears to permit the emergence of fusogenic variants during HIV-2 passage in vitro and may also regulate viral growth or cytopathic effects in selected cell types during natural infection in vivo.

Naturally occurring truncation of the transmembrane (TM) protein has been observed with isolates of human immunodeficiency virus type 2 (HIV-2) (1, 4, 8, 11, 17) and simian immunodeficiency virus of macaques (SIV_{mac}) (2, 3, 5, 12, 13, 16, 24) but not with isolates of HIV-1. Such viruses possess prematurely terminated *env* genes that encode TM subunits with short cytoplasmic domains: e.g., in HIV-2/ROD, the 154-amino-acid (154AA) cytoplasmic domain is truncated to 17AA (8). Interestingly, the TM subunits of lentiviruses normally possess cytoplasmic domains that are much longer than those of other retroviruses (e.g., that of HIV-1 is 152AA long, and that of Rous sarcoma virus is 30AA long) (7) and whose normal function is unknown. To date, direct studies of the effects of such naturally occurring cytoplasmic domain truncations on lentiviral glycoprotein structure or function have not been reported.

The present study was undertaken to investigate the glycoprotein regions responsible for differential syncytium formation by two genetically highly related but biologically distinct HIV-2 viruses. Recombinant vaccinia virus expression of the *env* gene of HIV-2/ST (ST) (a previously described replication-competent but noncytopathic virus [17, 18]) indicated that the glycoprotein was deficient in syncytium formation (22). The ST glycoprotein was also found to bind its cellular receptor CD4 with diminished affinity relative to HIV-1 and HIV-2 prototype viruses (14, 15, 22). Compared with wild-type ST, the *env* protein of a molecularly cloned cytopathic ST variant, designated HIV-2/ST/24.1C#2 (10), caused more efficient syncytium formation in the human T-cell line Sup T1, in which the variant was originally generated by repeated passage (23). In order to compare the primary structures of the cytopathic ST glycoprotein with that of the wild type, we determined the nucleotide sequence of the *env* gene of a representative molecular clone (10). The predicted AA sequence for the glycoprotein of cytopathic ST was found to differ by 11 residues from that of wild-type ST (18) (Fig. 1). Interestingly, the glycoprotein of cytopathic ST (hereafter designated ST#2) possessed five amino acid substitutions each in the external glycoprotein (gp120) and in the TM subunit and also a premature termination codon, which truncated the TM cytoplasmic domain by 147AA (Fig. 2A). By immunoblot analysis, the TM protein of ST#2 has been estimated to have a molecular mass of 32 kDa, compared with 41 kDa for wild-type ST (14).

To determine the specific glycoprotein domains or AA residues responsible for the enhanced fusion activity of the glycoprotein of ST#2, we constructed chimeric *env* genes, cloned them into vaccinia virus expression vectors, and evaluated expression and syncytium formation of the resulting envelope proteins in various CD4-bearing cell types. Two chimeric genes (A and B) contained wild-type ST sequences at their 5′ ends ligated to 3′ sequences of ST#2 at shared unique *Hae*II (chimera A) or *Nco*I (chimera B) restriction enzyme sites (nucleotides 7253 and 7952, respectively [18]). The resulting chimeric glycoproteins contained predicted N-terminal regions corresponding to wild-type ST and C-terminal regions representative of ST#2 (chimera A, 368AA ST, 344AA ST#2; chimera B, 601AA ST, 111AA ST#2) (Fig. 2B). Expression of the chimeric envelope glycoproteins was analyzed by metabolic labeling and immunoprecipitation (Fig. 2C). Following a 17-h chase period, the envelope glycoprotein precursors of ST and ST#2 of 160 and

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FIG. 1. Comparison of the envelope glycoprotein AA sequences of cytopathic ST#2 and wild-type ST. JSP4-27, wild-type ST; ST/24.1C#2, cytopathic ST#2. The single-letter AA code is used. Residues at which differences occur are in boldface; dashes indicate identity. #, premature TAA codon; SU, gp120 coding region; TM, transmembrane glycoprotein coding region. The ST/24.1C#2 sequence is truncated after AA712 by the premature termination codon.

140 kDa, respectively, were observed in the cell lysates (top band, lanes 1 and 3). The more rapid electrophoretic mobility of the ST#2 precursor resulted from its C-terminal truncation. The external glycoprotein (gp120) of ST (approximately 120 kDa) was observed in both the cell lysate (lower band, lane 1) and culture supernatant (lane 2), while the ST#2 gp120 was primarily seen in the culture supernatant (lane 4). Chimeric genes A and B each also possessed premature *env* termination and encoded precursor proteins (lanes 5 and 7, respectively) and gp120 proteins (best observed in supernatants, lanes 6 and 8, respectively) with electrophoretic patterns similar to that of ST#2. The presence of soluble gp120 proteins in cell culture media indicated that correct proteolytic processing and transport to cell surfaces occurred with both the parental and chimeric proteins. As described previously, the [^{35}S]methionine and [^{35}S]cysteine pulse-labeled TM subunits were not clearly resolved following immunoprecipitation of cell lysates (not shown) (2, 22).

We then compared syncytium formation by the vaccinia virus-expressed chimeric and parental glycoproteins in the human T-cell line Sup T1. ST#2 generated larger and more numerous syncytia than wild-type ST envelope protein (Fig. 3), as previously observed (23). To our surprise, chimeric proteins A and B each also formed larger and more numerous syncytia than wild-type ST (Fig. 2D and 3). These results indicated that efficient Sup T1 cell fusion by the ST#2 glycoprotein was determined by a C-terminal region of the TM subunit.

As defined by chimera B, the minimal relevant C-terminal region of the ST#2 TM protein comprised 111AA (AA 602 to 712 of Fig. 1). This region in ST#2 differs from that in wild-type ST at five residues, one of which is the premature termination codon which truncates the ST#2 cytoplasmic domain. To identify the specific AA alterations within this region that enhanced the fusion activity of the ST#2 glycoprotein, we performed site-directed mutagenesis (25) on the *env* gene of wild-type ST and initially tested for enhanced fusion activity with a T7 RNA polymerase expression system (6). To screen the potential AA determinants of enhanced fusion activity, we constructed mutant ST *env* genes coding for three of the AA residues at which ST#2 differed in the region of interest (positions G659→S, F712→S, and Q713→TAA stop codon). Two of these codons (712 and 713) were adjacent and so were most efficiently mutated with a single mutagenic primer (mutant 5). The other two AA differences between wild-type ST and ST#2 in this region of interest were very conservative (M615→I and I686→M) and were not evaluated. In the transient T7 system, the glycoprotein of mutant 5 but not the G659→S mutant induced significantly more syncytia in Sup T1 cells than wild-type ST (not shown). A vaccinia virus recombinant expressing the mutant 5 gene (Fig. 4A) was then constructed, and expression was evaluated by metabolic labeling and immunoprecipitation (Fig. 4B). Following the 17-h chase period, wild-type ST and ST#2 proteins (lanes 1 to 4) were observed as described above. The mutant 5 precursor protein of approximately 140 kDa was present in the cell lysate, whereas little gp120 was observed there (lane 5). The cell culture supernatant, however, contained soluble gp120 (lane 6), indicating that efficient processing and transport of the mutant protein had occurred. Continuous [^{35}S]methionine-[^{35}S]cysteine labeling of vaccinia virus recombinant-infected cells permitted visualization of the full-length or truncated TM proteins of wild-type ST and mutant 5, respectively (Fig. 4C). Strikingly, numerous large syncytia were generated by the mutant 5 glycoprotein in Sup T1 cells (Fig. 3D), similar to ST#2 and much greater than observed for wild-type ST glycoprotein

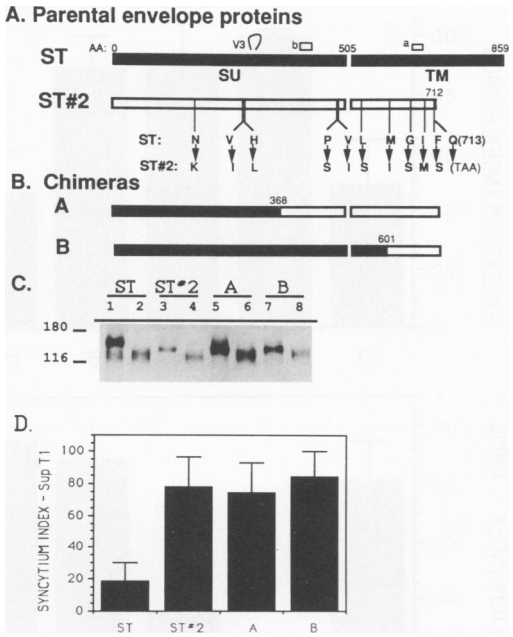

FIG. 2. Construction, expression, and fusion activity of the parental and chimeric HIV-2 envelope glycoproteins. (A) Parental glycoproteins: ST, HIV-2/ST.JSP4-27 (wild type); ST#2, HIV-2/ST/24.1C#2 (cytopathic variant); SU, external glycoprotein (gp120); TM, transmembrane glycoprotein (gp41 [ST] or gp32 [ST#2]); V_3, location of third variable loop of gp120; b, location of a CD4-binding epitope reported for HIV-1 (19); a, location of membrane-spanning anchor domain; Q(713) → (TAA) indicates the ST AA residue for which a stop codon is substituted in ST#2. The 11 AA residues that differ between ST and ST#2 are shown. (B) Construction of the chimeric glycoproteins. (C) Glycoprotein expression by the recombinant vaccinia viruses. HeLa cells were infected with the recombinants at a multiplicity of infection of 1 and pulse-labeled with [^{35}S]methionine-[^{35}S]cysteine, and cell lysates (odd-numbered lanes) or media (even-numbered lanes) were immunoprecipitated with human anti-HIV-2 serum following a 17-h chase period (22). Approximate molecular sizes (180 or 116 kDa) are shown to the left. (D) Syncytium assays in Sup T1 cells. A total of 7.5×10^4 cells (in a 96-well plate) were infected with the recombinants at a multiplicity of infection of 1 and visually scored for syncytium formation at 18 h postinfection as described previously (23). The syncytium index is calculated as [(number of syncytia observed)/(maximal number of syncytia, any recombinant)] × 100. Results are shown as mean syncytium index ± standard deviation for determinations by two observers, each scoring three independent experiments. In a representative experiment, the absolute numbers of syncytia per well observed for ST, ST#2, A, and B were 21, 210, 172, and 246, respectively.

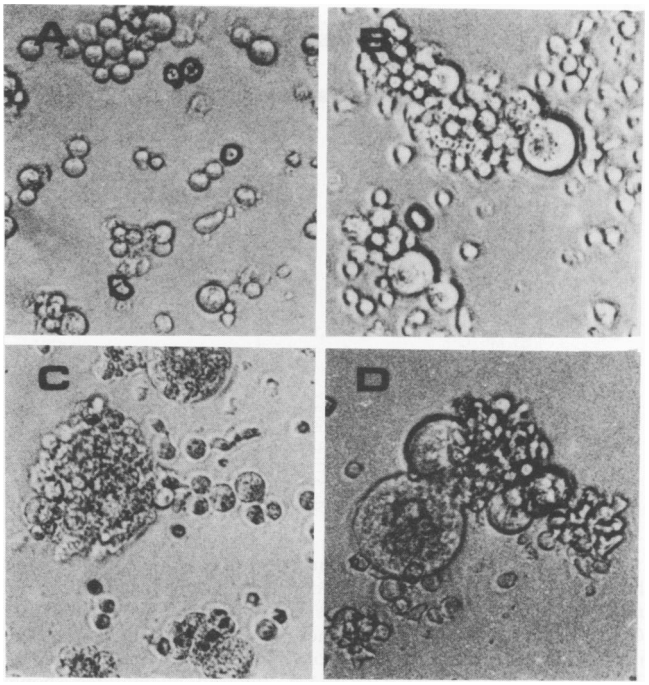

FIG. 3. Sup T1 cell fusion by the recombinant glycoproteins. Sup T1 cells infected with the vaccinia virus recombinants at a multiplicity of infection of 1 were photographed after 24 h of incubation. (A) ST; (B) ST#2; (C) chimera B; (D) mutant 5. The envelope glycoprotein of wild-type ST caused few, small syncytia, while that of ST#2, chimeras A (not shown) and B, and mutant 5 generated numerous, large syncytia.

(Fig. 4D). These results demonstrate that truncation of the cytoplasmic domain of the wild-type ST glycoprotein dramatically enhances syncytium formation in Sup T1 cells.

Previously we found that although the variant ST viruses arising during passage of wild-type ST in Sup T1 cells were fusogenic and cytopathic in that cell type, they remained, like ST, highly restricted in cell host range and failed to infect several CD4-bearing cell types (14). To investigate the effect of the cytoplasmic domain on fusion activity in cell types other than Sup T1, we again used the vaccinia virus expression system. In the human T-cell line Molt 4 clone 8, ST#2 was more efficient at inducing fusion than wild-type ST, although compared with the Sup T1 line, this cell type was more susceptible to wild-type ST fusion (Fig. 5A). Chimeras A and B fused Molt 4 clone 8 cells with the high efficiency of ST#2, while mutant 5 was no better at fusion of this cell type than wild-type ST. These results indicated that cytoplasmic domain truncation was not sufficient to account for the enhanced fusion activity of the ST#2 glycoprotein in the Molt 4 clone 8 cell line (unlike the result in Sup T1 cells). Rather, the results with chimera B suggested that efficient fusion of Molt 4 clone 8 cells by the ST#2 glycoprotein was encoded, either singly or cooperatively, among the five unique codons present in the ST#2 region of chimera B (positions 602 to 713) (Fig. 2A and B).

Previously, the envelope glycoprotein of wild-type ST but not ST#2 has been observed to cause detectable cell fusion of fresh human peripheral blood mononuclear cells (PBMCs) (23). We next evaluated the effect of cytoplasmic domain truncation on fusion activity in PBMCs (Fig. 5B) and found that the efficiency of chimera A was less than half that of wild-type ST, while chimera B fused PBMCs with an efficiency equal to that of wild-type ST. Mutant 5 generated syncytia with an efficiency no different from that of the wild-type ST glycoprotein. These results suggest that the cytoplasmic domain was neither a positive nor a negative modulator of glycoprotein fusion activity in human PBMCs. Furthermore, the results with chimeras A and B suggested that critical determinants of efficient PBMC fusion were encoded among the three unique AAs present between residues 369 and 601 of the wild-type ST glycoprotein (the ST region present in chimera B but not in chimera A) (Fig. 2A and B).

The above observations indicate that the cytoplasmic domain is capable of modulating the fusion activity of the

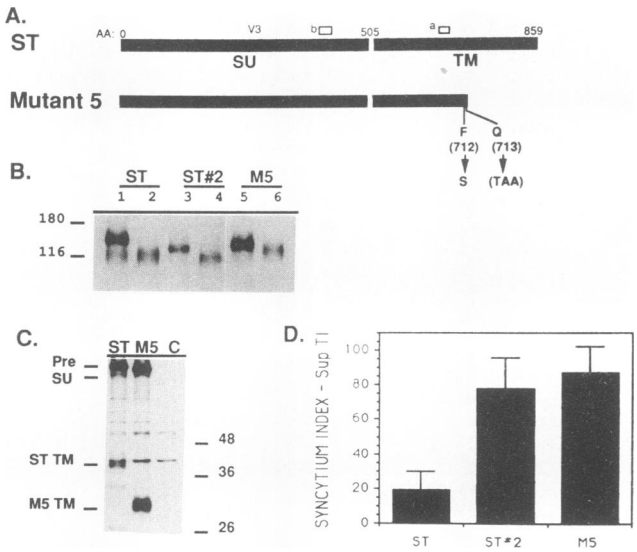

FIG. 4. Mutant 5 glycoprotein construction, expression, and fusion activity. (A) Mutant 5 glycoprotein is the ST glycoprotein mutated to reflect the premature termination codon (codon 713) and C-terminal residue (AA712) of the cytopathic variant ST#2. The mutagenic primer used in the construction of mutant 5 was 5′-TTAGGAGTACGCGGGGGGGGA-3′ (altered nucleotides shown in boldface), which is complementary to wild-type ST nucleotides 8267 to 8287 (18). (B) Expression of parental and mutant 5 glycoproteins by recombinant vaccinia viruses (method as in legend to Fig. 2C). (C) The TM proteins of wild-type ST and mutant 5 (M5) were observed following 6 h of continuous labeling and immunoprecipitation with cross-reactive rhesus monkey anti-SIV serum. C, control vaccinia virus recombinant. Several nonspecific bands (migrating between the surface [SU] and TM proteins) were also present in an overexposure of the control lane (not shown). Sizes are shown in kilodaltons. (D) Syncytium assays in Sup T1 cells (method and syncytium index as in legend to Fig. 2D).

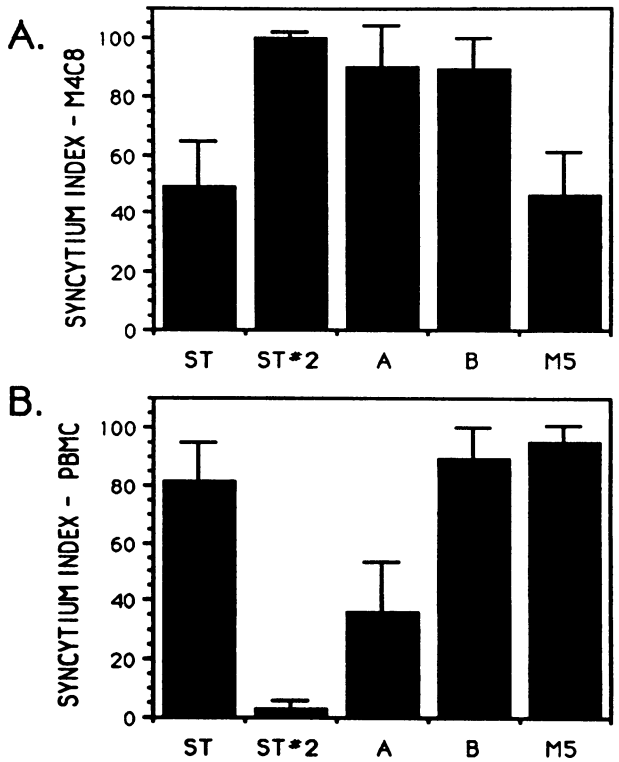

FIG. 5. Syncytium assays in two additional human cell types. Method and syncytium index are as described in the legend to Fig. 2D. A, Molt 4 clone 8 (M4C8) cells; B, human PBMCs (48-h phytohemagglutinin-stimulated blasts). M5, mutant 5.

exterior glycoprotein complex of HIV-2 in selected cell types. The observed differential effects of truncation of the cytoplasmic domain on fusion in three human cell types emphasize this cell specificity. Similarly, SIV_{sm}LIB1 was found to develop a truncated TM glycoprotein and faster replication kinetics during passage in the human T-cell line Hut 78 (20) but not in the CEMx174 line, in which the SIV_{sm} isolate initially replicated well (21). Although mutant 5 was a double mutant possessing the premature termination codon at position 713 and also a substitution at AA712, it is more likely that the striking Sup T1 cell fusion enhancement obtained with mutant 5 was due to the termination codon, since it caused a major structural alteration—loss of 147 AA—as opposed to the single AA substitution at position 712. Also supporting the view that the truncation itself, rather than the identity of the carboxy-terminal AA, was likely to have caused the enhanced fusion activity of mutant 5 are the reports that analogous site-directed mutants encoding premature *env* termination codons in SIV_{mac} provide a replicative advantage to that virus (perhaps via enhanced fusion activity, as our results would suggest) without a requirement for alteration of the carboxy-terminal residue (2, 12, 16).

While earlier mutagenesis studies with SIV_{mac} clearly indicated that mutations creating cytoplasmic domain truncations analogous to that found in ST#2 provided a selective growth advantage in certain human cell types, these studies did not further define the nature of the replicative advantage (2, 12, 16). In directly evaluating the effect of cytoplasmic domain truncation on glycoprotein function, we have shown that premature *env* termination in HIV-2 enhances the fusion activity of the glycoprotein in some types of cells. More efficient membrane fusion activity is likely to potentiate both viral entry and cell-to-cell transmission of viral genomes as well as the overall cytopathic potential of the virus. The precise biochemical mechanism by which alteration of the cytoplasmic domain of the HIV-2 glycoprotein affects the fusion activity of the exterior envelope complex is presently undefined. Possible explanations include altered glycoprotein export to the cell surface; greater lateral mobility of the glycoprotein in the lipid bilayer; distant conformational changes in exterior glycoprotein domains (i.e., which might enhance CD4 binding or fusion peptide function); and alteration of the noncovalent association between the exterior TM and gp120 molecules comprising the assembled glycoprotein complex. Interestingly, experimental HIV-1 cytoplasmic domain truncation has been shown to increase glycoprotein cleavage and export to the surface of CHO cells (9). Recently, the glycoprotein of an uncloned cytopathic ST strain possessing a truncated TM protein and, presumably, other AA changes relative to wild-type ST was shown to bind soluble CD4 with greater affinity than wild-type ST (14). Finally, whether HIV-2 strains possessing premature *env* terminations exist in infected humans in vivo is unknown, but the plasticity and adaptability of the cytoplasmic domain could have potentiated the ability of an SIV-like HIV-2 ancestor virus to extend its host range to humans.

Nucleotide sequence accession number. The nucleotide

sequence for the *env* gene of HIV-2/ST/24.1C#2 has been submitted to GenBank (accession number M86924).

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REFERENCES

1. **Albert, J., U. Bredberg, F. Chiodi, B. Bottiger, E. M. Fenyo, E. Norrby, and G. Biberfeld.** 1987. A new human retrovirus isolate of West African origin (SBL-6669) and its relationship to HTLV-IV, LAV-II, and HTLV-IIIB. AIDS Res. Hum. Retroviruses **3**:3–10.
2. **Chakrabarti, L., M. Emerman, P. Tiollais, and P. Sonigo.** 1989. The cytoplasmic domain of simian immunodeficiency virus transmembrane protein modulates infectivity. J. Virol. **63**:4395–4403.
3. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo.** 1987. Sequence from simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature (London) **328**:543–547.
4. **Evans, L. A., J. Moreau, K. Odehouri, H. Legg, A. Barboza, D. Cheng-Mayer, and J. A. Levy.** 1988. Characterization of a noncytopathic HIV-2 strain with unusual effects on CD4 expression. Science **240**:1522–1525.
5. **Franchini, G., C. Gurgo, H.-G. Guo, R. C. Gallo, E. Collati, K. A. Fargnoli, L. F. Hall, F. Wong-Staal, and M. S. Reitz, Jr.** 1987. Sequence of simian immunodeficiency virus and its relationship to human immunodeficiency viruses. Nature (London) **328**:539–543.
6. **Fuerst, T. R., E. G. Niles, R. W. Studier, and B. Moss.** 1986. Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. Proc. Natl. Acad. Sci. USA **83**:8122–8126.
7. **Gallaher, W. R., J. M. Ball, R. F. Garry, M. C. Griffin, and R. C. Montelaro.** 1989. A general model for the transmembrane proteins of HIV and other retroviruses. AIDS Res. Hum. Retroviruses **5**:431–440.
8. **Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) **326**:662–669.
9. **Haffar, O. K., G. R. Nakamura, and P. W. Berman.** 1990. The carboxy terminus of human immunodeficiency virus type 1 gp160 limits its proteolytic processing and transport in transfected cell lines. J. Virol. **64**:3100–3101.
10. **Hahn, B.** Unpublished data.
11. **Hasegawa, A., H. Tsujimoto, N. Maki, K.-I. Ishikawa, T. Miura, M. Fukasawa, K. Miki, and M. Hayami.** 1989. Genomic divergence of HIV-2 from Ghana. AIDS Res. Hum. Retroviruses **5**:593–604.
12. **Hirsch, V. M., P. Edmondson, M. Murphey-Corb, B. Arbeille, P. R. Johnson, and J. I. Mullins.** 1989. SIV adaption to human cells. Nature (London) **341**:573–574.
13. **Hirsch, V., N. Riedel, and J. I. Mullins.** 1987. The genome organization of STLV-3 is similar to that of the AIDS virus except for a truncated transmembrane protein. Cell **49**:307–319.
14. **Hoxie, J. A., L. E. Brass, C. H. Pletcher, B. S. Haggarty, R. Colloran, and B. Hahn.** 1991. Cytopathic variants of an attenuated isolate of human immunodeficiency virus type 2 exhibit increased affinity for CD4. J. Virol. **65**:5096–5101.
15. **Ivey-Hoyle, M., J. S. Culp, M. A. Chaikin, B. D. Hellmig, T. J. Matthews, R. W. Sweet, and M. Rosenberg.** 1991. Envelope glycoproteins from biologically diverse isolates of immunodeficiency viruses have widely different affinities for CD4. Proc. Natl. Acad. Sci. USA **88**:512–516.
16. **Kodama, T., D. P. Wooley, Y. M. Naidu, H. W. Kestler III, M. D. Daniel, Y. Li, and R. C. Desrosiers.** 1989. Significance of premature stop codons in *env* of simian immunodeficiency virus. J. Virol. **63**:4709–4714.
17. **Kong, L. I., S. Lee, J. C. Kappes, J. S. Parkin, D. Decker, J. A. Hoxie, B. H. Hahn, and G. M. Shaw.** 1988. West African HIV-2-related human retrovirus with attenuated cytopathicity. Science **240**:1525–1529.
18. **Kumar, P., H. Hui, J. C. Kappes, B. S. Haggarty, J. A. Hoxie, S. K. Arya, G. M. Shaw, and B. H. Hahn.** 1990. Molecular characterization of an attenuated human immunodeficiency virus type 2 isolate. J. Virol. **64**:890–901.
19. **Laskey, L. A., G. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. Cell **50**:975–985.
20. **Marx, P. A. (New Mexico Regional Primate Research Laboratory).** 1991. Personal communication.
21. **Marx, P. A., Y. Li, N. W. Lerche, S. Sutjipto, A. Gettie, J. A. Yee, B. H. Brotman, A. M. Prince, A. Hanson, R. G. Webster, and R. C. Desrosiers.** 1991. Isolation of a simian immunodeficiency virus related to human immunodeficiency virus type 2 from a West African pet sooty mangabey. J. Virol. **65**:4480–4485.
22. **Mulligan, M. J., P. Kumar, H. Hui, R. Owens, G. D. Ritter, Jr., B. H. Hahn, and R. W. Compans.** 1990. The *env* protein of an infectious noncytopathic HIV-2 is deficient in syncytium formation. AIDS Res. Hum. Retroviruses **6**:707–720.
23. **Mulligan, M. J., G. D. Ritter, Jr., M. A. Chaikin, G. V. Yamshchikov, P. Kumar, B. H. Hahn, R. W. Sweet, and R. W. Compans.** 1992. Human immunodeficiency virus type 2 envelope glycoprotein: differential CD4 interactions of soluble gp120 versus the assembled envelope complex. Virology **187**:233–241.
24. **Naidu, Y. M., H. W. Kestler III, Y. Li, C. V. Butler, D. P. Silva, D. K. Schmidt, C. D. Troup, P. K. Sehgal, P. Sonigo, M. D. Daniel, and R. C. Desrosiers.** 1988. Characterization of infectious molecular clones of simian immunodeficiency virus (SIV_{mac}) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIV_{mac}. J. Virol. **62**:4691–4696.
25. **Zoller, M., and M. Smith.** 1982. Oligonucleotide-directed mutagenesis using M130-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA. Nucleic Acids Res. **10**:6487–6500.