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Regions Required for CD4 Binding in the External Glycoprotein gp120 of Simian Immunodeficiency Virus

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The external domain of the envelope glycoprotein, gp120, of simian immunodeficiency virus (SIV) has been expressed as a mature secreted product using recombinant baculoviruses and the expressed protein, which has an observed molecular mass of 110 kDa, was purified by monoclonal antibody (MAb) affinity chromatography. N-terminal sequence analysis showed a signal sequence cleavage identity similar to that of the gp120s of both human immunodeficiency virus type 1 (HIV-1) and HIV type 2. The expressed molecule bound to soluble CD4 with an affinity that was approximately 10-fold lower than that of gp120 from HIV-1. A screening of the ability of SIV envelope MAbs to inhibit CD4 binding revealed two groups of inhibitory MAbs. One group is dependent on conformation, while the second group maps to a discrete epitope near the amino terminus. The particular role of the V3 loop region of the molecule in CD4 binding was investigated by the construction of an SIV-HIV hybrid in which the V3 loop of SIV was precisely replaced with the equivalent domain from HIV-1 MN. The hybrid glycoprotein bound HIV-1 V3 loop MAbs and not SIV V3 MAbs but continued to bind conformational SIV MAbs and soluble CD4 as well as the parent molecule.

Since its discovery and molecular characterization (6, 13, 23), simian immunodeficiency virus (SIV) has been used extensively as an animal model for human immunodeficiency virus (HIV) infection, particularly with a view to the development of a vaccine for the prevention of human AIDS. A number of reports have detailed the successful use of inactivated viral vaccines or infected cell preparations for the protection of primates from challenge with SIV (9). However, more recent findings suggest that the mechanism of protection in these experiments may be related to antibodies to cell components rather than to viral antigens (5, 41). In contrast, protection against infectious viral challenge in a related system, i.e., HIV infection of the chimpanzee, can be elicited solely by viral envelope preparations (1, 10) or by the passive transfer of anti-Env antibodies (8). Differences between the external envelope proteins of HIV and SIV have also been suggested following studies on the molecular basis of tropism and viral diversity. The most dramatic changes in the HIV envelope are largely, although not exclusively, associated with the V3 loop, while those in SIV are spread throughout the molecule (30, 40), although recent reports of the determinants of tropism in SIV have also mapped such sequences to the V3 loop region (14, 21). The identity of the principal neutralizing determinant on SIV Env also appears different from that of HIV-1 (17). Thus, although the sequence relatedness of the outer glycoproteins of HIV and SIV is clear (36), these results suggest that the conformation and/or presentation of the envelope to the host during infection may be different for HIV and SIV and indicate that further comparative studies on the biophysical properties of the envelope proteins of SIV are necessary in order to confirm the authenticity of SIV infection as a model for HIV. This is especially so when preparations for trial vaccine usage are considered, since it is increasingly clear, for both HIV and SIV, that the conformation of the surface (SU) glycoprotein can dramatically affect the antibody response elicited (7, 12, 17). Recently, evidence was presented that SIV and HIV envelope proteins differed in the sequences responsible for a fundamental property of the envelope, CD4 binding. Changes in the V3 loop of SIV were reported to abrogate CD4 binding (16), whereas the V3 sequences in HIV type 1 (HIV-1) have previously been shown to be wholly dispensable for binding to the receptor (31, 39, 46).

Here, we characterize a recombinant source of SIV gp120 and map the monoclonal antibodies (MAbs) to the SU domain that inhibit CD4 binding. We show that although some MAbs strongly inhibit CD4 binding, none of them maps to the V3 loop. Further, we confirm the lack of SIV V3 loop involvement in CD4 binding by describing a hybrid envelope glycoprotein in which the entire SIV loop is replaced with that of HIV-1 MN. This molecule continues to bind CD4 as well as the wild type.

Results and discussion. SIV gp120, a representative of the SIVmac251 strain, was expressed as a soluble secreted molecule by using a recombinant baculovirus generated by standard technologies (22). The product was identified in cell lysates and supernatants by Western blotting (immunoblotting) with MAbs (Fig. 1, lanes 1 and 3). The expression profile for this molecule was very similar to those we have described earlier for the expressed external envelope glycoproteins of HIV-1 (34) and HIV type 2 (HIV-2) (33). The protein had an observed molecular mass of 110 kDa, which was consistent with the addition of a high-mannose, low-complex-type carbohydrate typical of insect cell-expressed glycoprotein (32, 44). Cultures that were harvested late (more than 3 days postinfection) showed evidence of some glycoprotein cleavage, giving positively reactive antigen at 70 kDa (Fig. 1). We have not determined the exact site of cleavage; however, the size of the product formed would be consistent with nicking of the molecule in or around the V3 loop region, as has been observed for HIV-1 (4). SIV gp120 was purified to near homogeneity by MAb affinity column chromatography using MAb KK8 (19) essentially as described for HIV-1 gp120 (34), except that elu-

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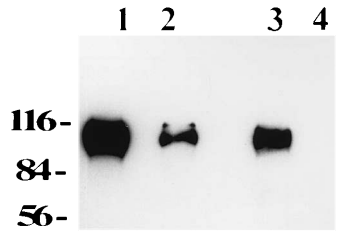

FIG. 1. Recombinant baculovirus expression of SIV gp120 wild type (lanes 1 and 3) and V3 loop-exchanged molecules (lanes 2 and 4) detected by Western blotting of infected-cell supernatants. *Spodoptera frugiperda* cells were infected at a multiplicity of infection of 10, and the supernatant was harvested 2 days postinfection. A 10-μl volume of supernatant was resolved on an SDS-10% polyacrylamide gel, and Western blotting was performed (2) using MAbs KK8 (lanes 1 and 2) and KK42 (lanes 3 and 4). Molecular masses in kilodaltons taken from prestained markers are listed on the left.

tion of the column was achieved using 50 mM diethylamine (pH 10.5), which was immediately neutralized by solid Tris sufficient to adjust the pH to 8.0. The purified protein was greater than 90% homogeneous, as judged by sodium dodecyl (SDS) sulfate-polyacrylamide gel electrophoresis (not shown) and was subjected to amino-terminal sequence analysis. The derived sequence was TQYVTVFYGVPAWR-ATIPL. This sequence starts at amino acid 23 of the predicted SIV gp120 coding sequence and formally identifies the signal cleavage site of the SIV SU glycoprotein as GIYC/TQYV, which is very similar to the LVYC/TQYV cleavage site that we previously reported for HIV-2 gp110 (33). Residue 15 was not identifiable in the sequence determination but is an asparagine in the predicted sequence that is part of an N-linked glycosylation site (NAT). This finding would be consistent with occupation of this site by a carbohydrate, as has been shown for HIV-1 gp120 (25).

CD4 binding by recombinant SIV gp120 was assayed by a capture enzyme-linked immunosorbent assay (ELISA) using the carboxy-terminal peptide serum D7368, which was similar to that previously described for the gp120s of HIV-1 and HIV-2 (26). Capture of SIV gp120 by the carboxy terminus did not alter the pattern of polyvalent serum binding to the envelope (28) or the binding of a panel of SIV envelope MAbs (data not shown). When it was directly compared with equivalent quantities of HIV-1 gp120 expressed in the same system (34), SIV gp120 was approximately 10-fold less active in binding receptor (data not shown). This figure is very similar to the value described for gp110 of HIV-2 (27) for the virion-derived SIV envelope (28) and is also consistent with the higher levels of soluble CD4 required to block SIV, as opposed to HIV, infection in culture (3). Seventeen MAbs raised against the SIV envelope (19) were purified and screened at saturating concentration for their ability to block the gp120-soluble CD4 interaction. Four MAbs, KK3, KK18, KK44, and KK56, were shown to completely prevent CD4 binding by SIV gp120 (Fig. 2), whereas many MAbs, including those directed to the V3 loop, partially reduced the level of gp120-CD4 binding. The pattern of inhibition shown by each MAb in the single-point assay was maintained after titration of each antibody (Fig. 3), and only the four MAbs originally identified showed complete abrogation of CD4 binding. MAbs KK44 and KK56 are neutralizing (18), recognize a conformational epitope, and compete with all MAbs in the previously defined competition group 1 (17a, 20). MAbs KK3 and KK18 have no neutralizing activity and belong to a separate competition group (group 4) that has been mapped to the peptide YCTQYVTVFYGVP AWRNATI (20). These data indicate that epitope occlusion by

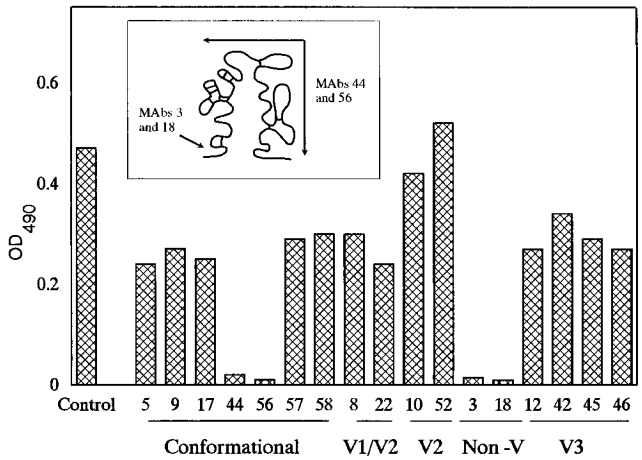

FIG. 2. Screen of 17 SIV Env MAbs for their abilities to inhibit CD4 binding by SIV gp120. Soluble CD4 at 2 μg/ml was captured to the solid phase by using MAb L120.3 (15) and was incubated with tissue culture supernatants containing SIV gp120, which had been previously incubated overnight at 4°C with 100 μg of each of the KK MAbs shown per ml. SIV gp120 glycoprotein was allowed to bind to CD4 for 2 h at room temperature (20°C) before being washed and detected with D7368 as described elsewhere (28). The readings are the means of duplicate wells. The control well received no antibody. (Inset) Locations of the epitopes for MAbs that inhibit CD4 binding. KK44 and KK56 are conformational epitopes and are indicated as binding to the whole gp120 structure. KK3 and KK18 bind to a distinct amino-terminal epitope, YCTQYVTVFYGVPAWRNATI. OD_{490}, optical density at 490 nm.

MAbs at either of two distinct regions of SIV gp120 (see inset in Fig. 2) leads to inhibition of receptor binding. Partial inhibition of the binding reaction seems likely to be due to steric hindrance but not direct occlusion of the CD4-gp120 site of contact. The identity of conformational MAbs KK44 and KK56 as inhibitory is consistent with a number of MAbs with similar properties that have been reported for HIV-1 (see, e.g., reference 43), although it should be noted that MAbs KK9 and KK5, which also mapped to competition group 1, did not occlude CD4 binding. Recent mapping data have shown that both KK9 and KK5 are distinct from KK44 and KK56. The binding of KK9 to SIV Env is uniquely effected by sequence changes in the V3 loop, and KK5 is the only MAb in this group that binds to SIVsm7 in addition to SIVmac251 (18). Thus, the properties of KK44 and KK56 mark them as a distinct subgroup within the group 1 MAbs (group 1C), which is consistent with their sole ability to inhibit the gp120-CD4 binding reac-

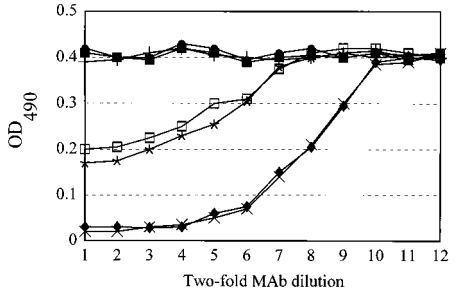

FIG. 3. Titration of the MAbs that affect CD4 binding. Two MAbs were selected from each class of response shown in Fig. 2 (i.e., no inhibition, partial inhibition, or full inhibition), were diluted in twofold steps from an initial concentration of 50 μg/ml, and were incubated with SIV gp120 at 1 μg/ml overnight. CD4 binding was subsequently assayed as described in the legend Fig. 2. To MAb symbols: ■, control (no MAb addition); ●, KK10; ◆, KK56; X, KK44; □, KK42; +, KK52; ❊, KK17. OD_{490}, optical density at 490 nm.

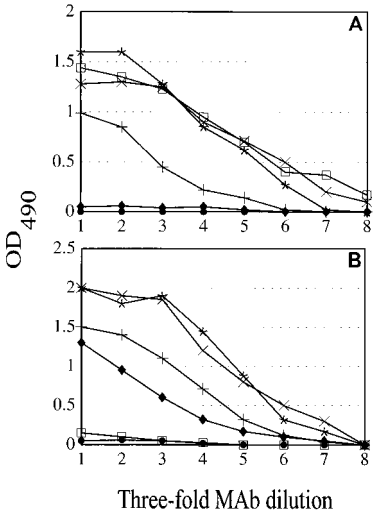

FIG. 4. ELISA characterization of hybrid SIV gp120 containing the HIV-1 MN V3 loop. Parental (A) or V3 loop exchange (B) glycoproteins were captured to the solid phase using D7368 and were incubated with threefold dilutions of MAb to SIV Env or to the MN V3 loop (MAb ADP4033) beginning at 25 μg/ml. MAb binding was detected by an anti-mouse alkaline phosphatase conjugate and AMPAK (28). MAb symbols: ●, control; □, KK42; ◆, ADP4033; ❄, KK19; +, KK3; X, KK56. OD_{490}, optical density at 490 nm.

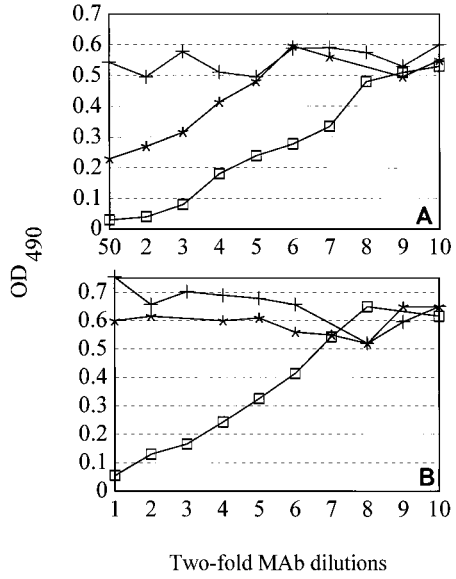

FIG. 5. CD4 binding by V3 loop-exchanged glycoprotein in the presence of inhibitory MAbs. The CD4-binding assay and preincubations with MAbs were done as described in the legends to Fig. 2 and 3, with an initial MAb concentration of 50 μg/ml. +, control (no MAb addition); □, KK56; ❄, KK42. (A) Parental SIV gp120; (B) the SIV gp120 (V3 MN). OD_{490}, optical density at 490 nm.

tion. The epitope for MAbs KK3 and KK18 in the amino-terminal region of the molecule is not normally considered part of the CD4-binding site of HIV-1 gp120, since mutations in this region have only a marginal effect on CD4 binding (37). However, an antiserum raised against HIV gp120 amino acids 42 to 129, part of which overlaps the sequence analogous to that of the KK3-KK18 epitope, has been reported to inhibit CD4 binding (42). Taken together, these data strongly suggest that the regions of SIV gp120 that are involved in CD4 binding are the structural equivalents of the sequences previously mapped as important in HIV-1 gp120. Since the finding that MAbs to the V3 loop failed to prevent CD4 binding did not agree with the report by Javarherian et al. (16), we sought to examine the role of SIV V3 loop sequences in CD4 binding by a direct assay. Accordingly, a hybrid SIV glycoprotein was constructed in which the V3 loop was replaced cysteine to cysteine with the equivalent loop from HIV-1 MN. As a consequence of this construction, the sequence identity of the loop was completely altered, although we cannot rule out the possibility that some essential features of the loop were maintained despite the sequence exchange. Following expression of the hybrid glycoprotein using recombinant baculoviruses, the antigenic structure of the molecule was assessed by Western blots and ELISA. The loop exchange molecule (SIV gp120 V3 MN) reacted in Western blotting with the V1-V2 loop MAb KK8 but not with the SIV V3 loop-specific MAb KK42 (Fig. 1, lanes 2 and 4). Similarly, in ELISAs, exchange of the loop did not alter the binding of the conformational MAb KK56, yet binding of the V3 loop MAb KK42 was abolished and replaced by positive binding by MN V3 loop-specific MAb ADP4033 (Fig. 4). CD4 binding was assessed for the hybrid glycoprotein in the presence of a subset of MAbs that prevented binding of the wild-type molecule. The hybrid glycoprotein bound CD4 as well as the parental molecule and was similarly inhibited by KK44 (Fig. 5). In contrast to the parental molecule, there was no partial inhibition of CD4 binding by the hybrid in the presence of KK42, as expected from the sequence changes made.

Earlier studies of SIV envelope expressed in mammalian cells showed the molecule to be in a conformation suitable for CD4 binding and to elicit neutralizing antibody; however, a map of the regions of the molecule involved in CD4 binding was not reported (38). More recent mapping experiments suggested that sequences involved in CD4 binding were located in the SIV V3 loop (16), in stark contrast to the results available for HIV-1 (31, 39, 46). As far as we have determined, the structural properties of SIV gp120 expressed by recombinant baculoviruses appear to be very similar to those previously described for the SU glycoproteins of HIV-1 and HIV-2 and reinforce the idea, which has been primarily derived from sequence comparisons, that the overall conformation of SIV gp120 is very similar to that of HIV gp120. The finding that antibodies to equivalent regions of the molecule block receptor binding is a particularly strong indicator of a structure-function relationship shared between the two molecules. In our assays, MAbs to the V3 loop of SIV gp120 diminished but did not abolish CD4 binding, a result that was confirmed by the exchange of the V3 loop of SIV for that of HIV-1 MN without loss of CD4 binding. The discrepancy between our results and those of Javaherian et al. (16) remains unclear, although there is confirmatory evidence in support of a link between V3 loop identity and the biochemical properties of the envelope (45). Moreover, for both HIV and SIV gp120s, there is a clear and close link between the V3 domain and the CD4-binding site since, in both systems, mutants in the CD4-binding domain can be compensated by mutations in V3 (29, 35, 47). The CD4-binding assays reported by Javaherian et al. were based on immune precipitation and so were, in essence, single-point assays. Since the binding of KK42 to SIV gp120 lessened the CD4 binding reaction, it is possible that at the concentrations used by Javaherian et al., reduction in binding was sufficient to score as nonbinding. Similarly, in their work only the sequence identity at the crown of the V3 loop was changed. The loop has been predicted to form a distinct three-dimensional structure

that is shared between many strains despite sequence variation (11, 24). It is possible that, at least in SIV, the relevant structure must be preserved for the molecule to function. A partially changed V3 loop may not allow such a structure to form, whereas a complete exchange may successfully substitute the necessary structure despite the changed character of the primary sequence involved.

Since we conclude that the gross aspects of envelope conformation are shared by the gp120s of HIV-1 and SIV, it remains difficult to explain the apparently altered presentation of the variable domains of SIV gp120 to the host compared with those of HIV gp120 (17). However, these findings may reflect the altered properties of the higher-order structure of the envelope (as reported for HIV-1 [7]) rather than differences in conformation measured in soluble, largely monomeric gp120 antigen. Further studies of the envelope of each virus will be necessary to understand this aspect of conformation and to provide additional data for the development of rationally designed candidate vaccines.

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REFERENCES

1. **Berman, P. W., T. J. Gregory, L. Riddle, G. R. Nakamura, M. A. Champe, J. P. Porter, F. M. Wurm, R. D. Hershberg, E. K. Cobb, and J. W. Eichberg.** 1990. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature (London) **345:** 622–625.
2. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulphate polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112:**195–203.
3. **Clapham, P. R., J. N. Weber, D. Whitby, K. McIntosh, A. G. Dalgleish, P. J. Maddon, K. C. Deen, R. W. Sweet, and R. A. Weiss.** 1989. Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. Nature (London) **337:**368–370.
4. **Clements, G. J., M. J. Price-Jones, P. E. Stephens, C. Sutton, T. F. Schulz, P. R. Clapham, J. A. McKeating, M. O. McClure, S. Thomson, M. Marsh, J. Kay, R. A. Weiss, and J. P. Moore.** 1991. The V3 loops of the HIV-1 and HIV-2 surface glycoproteins contain proteolytic cleavage sites: a possible function in viral fusion? AIDS Res. Hum. Retroviruses **7:**3–16.
5. **Cranage, M. P., A. Baskerville, L. A. Ashworth, M. Dennis, N. Cook, S. Sharpe, G. Farrar, J. Rose, P. A. Kitchin, and P. J. Greenaway.** 1992. Intrarectal of macaques vaccinated with formalin-inactivated simian immunodeficiency virus. Lancet **339:**273–274.
6. **Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers.** 1985. Isolation of T-cell tropic HTLV-III like retrovirus from macaques. Science **228:**1201–1204.
7. **Earl, P. L., C. C. Broder, D. Long, S. A. Lee, J. Peterson, S. Chakrabarti, R. W. Doms, and B. Moss.** 1994. Native oligomeric human immunodeficiency virus type 1 envelope glycoprotein elicits diverse monoclonal antibody reactivities. J. Virol. **68:**3015–3026.
8. **Emini, E. A., W. A. Schleif, J. H. Nunberg, A. J. Conley, Y. Eda, S. Tokiyoshi, S. D. Putney, S. Matsushita, K. E. Cobb, C. M. Jett, J. W. Eichberg, and K. K. Murphy.** 1992. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. Nature (London) **355:**728–730.
9. **Gardner, M.** 1991. SIV vaccines: current status. The role of the SIV-macaque model in AIDS research. Vaccine **9:**787–791.
10. **Girard, M., M. P. Kieny, A. Pinter, F. Barre-Sinoussi, P. Nara, H. Kolbe, K. Kusumi, A. Chaput, T. Reinhart, E. Muchmore, et al.** 1991. Immunization of chimpanzees confers protection against challenge with human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **88:**542–546.
11. **Gupta, G., G. M. Anantharamaiah, D. R. Scott, J. H. Eldridge, and G. Myers.** 1993. Solution structure of the V3 loop of a Thailand HIV isolate. J. Biomol. Struct. Dyn. **11:**345–366.
12. **Haigwood, N. L., P. L. Nara, E. Brooks, G. A. Van-Nest, G. Ott, K. W. Higgins, N. Dunlop, C. J. Scandella, J. W. Eichberg, and K. S. Steimer.** 1992. Native but not denatured recombinant human immunodeficiency virus type 1 gp120 generates broad-spectrum neutralizing antibodies in baboons. J. Virol. **66:**172–182.
13. **Hirsch, V., N. Riedel, H. Kornfeld, P. J. Kanki, M. Essex, and J. I. Mullins.** 1986. Cross-reactivity to human T-lymphotropic virus type III/lymphadenopathy associated virus and molecular cloning of simian T-cell lymphotropic virus type III from African green monkeys. Proc. Natl. Acad. Sci. USA **83:**9754–9758.
14. **Hirsch, V. M., J. E. Martin, G. Dapolito, W. R. Elkins, W. T. London, S. Goldstein, and P. R. Johnson.** 1994. Spontaneous substitutions in the vicinity of the V3 analog affect cell tropism and pathogenicity of simian immunodeficiency virus. J. Virol. **68:**2649–2661.
15. **Holmes, H. C. (ed.)** 1991. AIDS reagent project: catalogue of reagents. Supplement 1991–1992. Medical Research Council AIDS Reagent Project, National Institute for Biological Standards and Control, Potters Bar, United Kingdom.
16. **Javaherian, K., A. J. Langlois, D. C. Montefiori, K. A. Kent, K. A. Ryan, P. D. Wyman, J. Stott, D. P. Bolognesi, M. Murphy-Corb, and G. J. Larosa.** 1994. Studies of the conformation-dependent neutralizing epitopes of simian immunodeficiency virus envelope protein. J. Virol. **68:**2624–2631.
17. **Javaherian, K., A. J. Langlois, S. Schmidt, M. Kaufmann, N. Cates, J. P. Langedijk, R. H. Meloen, R. C. Desrosiers, D. P. W. Burns, D. P. Bolognesi, G. L. LaRosa, and S. D. Putney.** 1992. The principal neutralization determinant of simian immunodeficiency virus differs from that of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **89:**1418–1422.

17a. **Kent, K.** Unpublished observations.

18. **Kent, K., C. Powell, T. Corcoran, A. Jenkins, N. Almond, I. Jones, J. Robinson, and J. Stott.** 1993. Characterisation of neutralising epitopes on simian immunodeficiency virus envelope using monoclonal antibodies. Proceedings of the 8th Colloque des Cent Gardes. Institut Pasteur, Paris.
19. **Kent, K. A., L. Gritz, G. Stallard, M. P. Cranage, C. Collignon, C. Thiriart, T. Corcoran, P. Silvera, and E. J. Stott.** 1991. Production and characterisation of monoclonal antibodies to simian immunodeficiency virus envelope glycoproteins. AIDS **5:**829–836.
20. **Kent, K. A., E. Rud, T. Corcoran, C. Powell, C. Thiriart, C. Collignon, and E. J. Stott.** 1992. Identification of two neutralizing and 8 non-neutralizing epitopes for simian immunodeficiency virus envelope using monoclonal antibodies. AIDS Res. Hum. Retroviruses **8:**1147–1151.
21. **Kirchhoff, F., K. Mori, and R. C. Desrosiers.** 1994. The "V3" domain is a determinant of simian immunodeficiency virus cell tropism. J. Virol. **68:** 3682–3692.
22. **Kitts, P., M. Ayres, and R. Possee.** 1991. Linearisation of baculovirus DNA improves the selection of recombinant transfer vectors. Nucleic Acids Res. **18:**5667–5672.
23. **Kornfeld, H., N. Riedel, G. A. Viglianti, V. Hirsch, and J. I. Mullins.** 1987. Cloning of HTLV-4 and its relation to simian and human immunodeficiency viruses. Nature (London) **326:**610–613.
24. **LaRosa, G. J., J. P. Davide, K. Weinhold, J. A. Waterbury, A. T. Profy, J. A. Lewis, A. J. Langlois, G. R. Dreesman, R. N. Boswell, P. Shadduck, et al.** 1990. Conserved sequence and structural elements in the HIV-1 principal neutralizing determinant. Science **249:**932–935.
25. **Leonard, C. K., M. W. Spellman, L. Riddle, R. J. Harris, J. N. Thomas, and T. J. Gregory.** 1990. Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. J. Biol. Chem. **265:**10373–10382.
26. **Moore, J. P.** 1990. Simple methods for monitoring HIV-1 and HIV-2 gp120 binding to soluble CD4 by enzyme-linked immunosorbent assay: HIV-2 has a 25-fold lower affinity than HIV-1 for soluble CD4. AIDS **4:**297–305.
27. **Moore, J. P., Y. Morikawa, and I. M. Jones.** 1991. Binding of HIV-1 and HIV-2 surface glycoproteins to soluble CD4. Acquired Immune Defic. Syndr. **4:**442–449.
28. **Moore, J. P. M., and Q. J. Sattentau.** 1993. Detecting SIV gp120 and its interaction with soluble CD4 by ELISA. AIDS Res. Hum. Retroviruses **12:**1297–1299.
29. **Moore, J. P. M., M. Thali, B. A. Jameson, F. Vignaux, G. K. Lewis, S.-W. Poon, M. Charles, M. S. Fung, B. Sun, P. J. Durda, L. Akerblom, B. Wahren, D. D. Ho, Q. J. Sattentau, and J. Sodroski.** 1993. Immunochemical analysis of the gp120 surface glycoprotein of human immunodeficiency virus type 1: probing the structure of the C4 and V4 domains and the interaction of the C4 domain with the V3 loop. J. Virol. **67:**4785–4796.
30. **Mori, K., D. J. Ringler, T. Kodama, and R. C. Desrosiers.** 1992. Complex determinants of macrophage tropism in *env* of simian immunodeficiency virus. J. Virol. **66:**2067–2075.
31. **Morikawa, Y., J. P. Moore, E. Fenouillet, and I. M. Jones.** 1992. Complementation of human immunodeficiency virus glycoproteins in trans. J. Gen. Virol. **73:**1907–1913.
32. **Morikawa, Y., J. P. Moore, and I. M. Jones.** 1990. HIV-1 envelope glycoprotein gp120 expression in *E. coli*: assessment of CD4 binding and use in epitope mapping. J. Virol. Methods **29:**105–114.
33. **Morikawa, Y., J. P. Moore, A. J. Wilkinson, and I. M. Jones.** 1991. Reduction in CD4 binding affinity associated with removal of a single glycosylation site in the external glycoprotein of HIV-2. Virology **180:**853–856.
34. **Morikawa, Y., H. A. Overton, J. P. Moore, A. J. Wilkinson, R. L. Brady, S. J. Lewis, and I. M. Jones.** 1990. Expression of HIV-1 gp120 and human soluble

CD4 by recombinant baculoviruses and their interaction in vitro. AIDS Res. Hum. Retroviruses **6:**765–773.
35. **Morrison, H. G., F. Kirchhoff, and R. C. Desrosiers.** 1993. Evidence for the cooperation of gp120 amino acids 322 and 448 in SIVmac entry. Virology **195:**167–174.
36. **Myers, G. L., B. Korber, S. Wain-Hobson, R. F. Smith, and G. N. Pavlakis (ed.).** 1993. Human retroviruses and AIDS 1993. Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, N.Mex.
37. **Olshevsky, U., E. Helseth, C. Furman, J. Li, W. Haseltine, and J. Sodroski.** 1990. Identification of individual human immunodeficiency virus type 1 gp120 amino acids important for CD4 receptor binding. J. Virol. **64:**5701–5707.
38. **Planelles, V., N. L. Haigwood, M. L. Marthas, K. A. Mann, C. Scandella, W. D. Lidster, J. R. Shuster, R. Van-Kuyk, P. A. Marx, M. B. Gardner, and P. A. Luciw.** 1991. Functional and immunological characterization of SIV envelope glycoprotein produced in genetically engineered mammalian cells. AIDS. Res. Hum. Retroviruses **7:**889–897.
39. **Pollard, S. R., M. D. Rosa, J. J. Rosa, and D. C. Wiley.** 1992. Truncated variants of gp120 bind CD4 with high affinity and suggest a minimum CD4 binding region. EMBO J. **11:**585–591.
40. **Sakai, H., S. Sakuragi, J.-I. Sakuragi, M. Kawamura, R. Shibata, and A. Adachi.** 1992. Sequences responsible for efficient replication of simian immunodeficiency virus SIVMND in cells of the monocyte/macrophage lineage. J. Gen. Virol. **73:**2989–2993.
41. **Stott, E. J.** 1991. Anti-cell antibody in macaques. Nature (London) **353:**393.
42. **Syu, W. J., J. H. Huang, M. Essex, and T. H. Lee.** 1990. The N-terminal region of the human immunodeficiency virus envelope glycoprotein gp120 contains potential binding sites for CD4. Proc. Natl. Acad. Sci. USA **87:**3695–3699.
43. **Thali, M., C. Furman, D. D. Ho, J. Robinson, S. Tilley, A. Pinter, and J. Sodroski.** 1992. Discontinuous, conserved neutralization epitopes overlapping the CD4-binding region of human immunodeficiency virus type 1 gp120 envelope glycoprotein. J. Virol. **66:**5635–5641.
44. **Wells, D. E., and R. W. Compans.** 1990. Expression and characterization of a functional human immunodeficiency virus envelope glycoprotein in insect cells. Virology **176:**575–586.
45. **Willey, R. L., T. S. Theodore, and M. A. Martin.** 1994. Amino acid substitutions in the human immunodeficiency virus type 1 gp120 V3 loop that change viral tropism also alter physical and functional properties of the virion envelope. J. Virol. **68:**4409–4419.
46. **Wyatt, R., N. Sullivan, M. Thali, H. Repke, D. Ho, J. Robinson, M. Posner, and J. Sodroski.** 1993. Functional and immunological characterization of human immunodeficiency virus type 1 envelope glycoproteins containing deletions of the major variable loops. J. Virol. **67:**4557–4565.
47. **Wyatt, R., M. Thali, S. Tilley, A. Pinter, M. Posner, D. Ho, J. Robinson, and J. Sodroski.** 1992. Relationship of the human immunodeficiency virus type 1 gp120 third variable loop to a component of the CD4 binding site in the fourth conserved region. J. Virol. **66:**6997–7004.