JOURNAL OF VIROLOGY, Sept. 1992, p. 5610–5614
0022-538X/92/095610-05$02.00/0

Vol. 66, No. 9

# Multimeric CD4 Binding Exhibited by Human and Simian Immunodeficiency Virus Envelope Protein Dimers

PATRICIA L. EARL,[1]* ROBERT W. DOMS,[1,2]† AND BERNARD MOSS[1]

*Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases,[1] and Laboratory of Pathology, National Cancer Institute,[2] Bethesda, Maryland 20892*

Received 18 March 1992/Accepted 28 May 1992

**The envelope (Env) glycoproteins of human and simian immunodeficiency viruses (HIV and SIV) form noncovalently associated oligomers which mediate virus binding to the cell surface and fusion between the viral envelope and plasma membrane. A high-affinity interaction with CD4 is a critical step in this process. In this report, we show that Env protein dimers, but not monomers, can bind two CD4 molecules simultaneously. Multimeric CD4 binding may have important implications for Env protein-CD4 avidity, CD4-induced release of gp120, and subunit-subunit cooperativity during virus membrane fusion as well as for therapeutic strategies.**

The oligomeric envelope (Env) glycoprotein of human immunodeficiency virus type 1 (HIV-1) mediates binding of the virus to the cell surface as well as fusion between the viral envelope and plasma membrane (20). The postbinding events which lead to membrane fusion are poorly understood but presumably include a conformational change in the Env protein which exposes the hydrophobic amino terminus of gp41, which has been implicated as playing a critical role in the fusion reaction (5, 12, 17, 19). The possibility that CD4 binding triggers this conformational change has been investigated by a number of groups. Indeed, the binding of CD4 to Env has been found to induce shedding of gp120 in some viral strains as well as to cause other structural alterations (2, 4, 13, 15, 22–24, 32). However, it has been questioned whether a single CD4 binding event is sufficient to cause fusion. Moore and coworkers have suggested that CD4-induced dissociation of gp120 occurs only when both Env subunits in a dimer bind CD4 and that multimeric binding may be a component in the virus-cell fusion reaction (23). Layne et al. found positive synergy in blocking infection of HIV-1 with soluble CD4 and speculated that while a single gp120-CD4 binding event may be sufficient for initiating virus binding to the cell surface, adsorption and membrane fusion may require multiple gp120-CD4 interactions (18). To explore the feasibility of these proposals, we have extended our work on Env protein oligomerization to examine whether Env protein oligomers exhibit multimeric CD4 binding.

To determine whether env protein oligomers exhibit multimeric CD4 binding, we developed a coprecipitation assay (Fig. 1). CD4 contains a ~370-amino-acid ectodomain composed of four immunoglobulinlike regions. Mutational studies as well as studies with truncated derivatives of CD4 have shown that the first N-terminal domain is critical for binding gp120 (30, 35). As a result, truncated forms of CD4 which lack the transmembrane domain but contain at least the first immunoglobulinlike domain are secreted from cells and can bind gp120 with high affinity. Two such molecules which have been expressed from recombinant vaccinia viruses are termed sCD4-177 and sCD4-372 (3, 21). The former contains the first two immunoglobulinlike domains (177 residues), while the latter contains all four (372 residues). While both forms of sCD4 bind gp120 with high affinity, they are antigenically distinct. A commercially available antibody, OKT4, recognizes sCD4-372 but not sCD4-177, while a second antibody, OKT4A, recognizes both.

We reasoned that if equimolar amounts of sCD4-177 and sCD4-372 were incubated with monomeric gp120, OKT4 would immunoprecipitate sCD4-372 and the Env protein to which it is bound. Neither free nor Env-bound sCD4-177 should be immunoprecipitated under these conditions. However, if oligomeric forms of Env protein were used, then coprecipitation of sCD4-177 by OKT4 may ensue if Env protein dimers and higher-order forms are capable of binding two or more CD4 molecules (Fig. 1). If so, then a significant proportion of Env protein dimers (up to 50%) would be expected to bind one sCD4-372 and one sCD4-177 molecule, assuming that equimolar amounts of each are used. Only if multimeric binding occurs will OKT4 be able to immunoprecipitate sCD4-177.

To produce HIV-1 Env protein, we infected cells with a recombinant vaccinia virus which expresses the full-length Env protein from the BH8 isolate (26). However, because CD4 binding can induce dissociation of gp120 from gp41 (4, 13, 15, 24), we used a modified form of the Env protein which is not cleaved into gp120 and gp41 subunits (10). This protein, expressed by recombinant virus vPE12, folds, assembles, binds CD4, and is transported normally (11). Approximately 16 h postinfection (p.i.), cells expressing Env were washed twice with phosphate-buffered saline (PBS), after which media containing different ratios of metabolically labeled sCD4-177 and sCD4-372 were added. The amount of sCD4 added was well in excess of the amount needed to saturate cell surface Env protein. After binding was allowed to proceed for 3 h at 37°C, the cells were washed twice with PBS and lysed with Triton X-100, and aliquots were immunoprecipitated with OKT4 or anti-Env antibody. As seen in Fig. 2A, the anti-Env antibody coprecipitated both forms of sCD4 from cells expressing Env (lanes 1 to 3) but not from cells infected with a control virus (lane 13), confirming that both sCD4 molecules bind to HIV-1 Env. Furthermore, the relative amounts of sCD4-372 and sCD4-177 coprecipitated by anti-Env (determined by scanning densitometry) accurately reflected the ratios added to the media. Thus, when equivalent amounts of sCD4-372 and sCD4-177 were added

* Corresponding author.
† Present address: Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

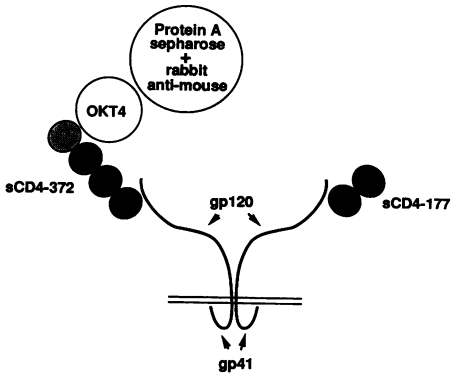


FIG. 1. Immunoprecipitation assay to detect multimeric CD4 binding. An uncleaved Env molecule is depicted in dimeric form spanning a membrane. One molecule each of sCD4-372 and sCD4-177, which contain four and two of the immunoglobulin G-like domains, respectively, are shown bound to the external gp120 portions of the env dimer. The OKT4 monoclonal antibody is shown bound to the fourth immunoglobulin G-like domain of sCD4-372. Only if a multimeric complex such as this is formed will OKT4 coprecipitate sCD4-177.

to the media, equivalent amounts bound to cell surface Env. These results suggest that the affinities of sCD4-177 and sCD4-372 for Env are approximately equal.

To determine whether the Env protein exhibited multimeric CD4 binding, aliquots of the cell lysates were immunoprecipitated with OKT4, which recognizes sCD4-372 but not sCD4-177. When cells were incubated with sCD4-372 alone, the amount of sCD4-372 immunoprecipitated by OKT4 (Fig. 2A, lane 8) was identical to the amount coprecipitated by anti-Env (lane 11), showing that only Env-bound sCD4 remained cell associated after washing and that little dissociation of sCD4 from Env occurred during immunoprecipitation. As predicted, OKT4 was unable to coprecipitate sCD4-177 from lysates of cells which had been incubated with sCD4-177 alone (lane 7). However, when cells were incubated with both sCD4-372 and sCD4-177, OKT4 immunoprecipitated both molecules, demonstrating that Env protein is capable of binding multiple CD4 molecules (lanes 4 to 6). Since most cell surface Env is present in dimeric form (9) and the affinities of sCD4-177 and sCD4-372 for Env are similar, we could predict the amount of sCD4-177 which should be coprecipitated by OKT4 at each of the three sCD4 ratios used. We found that approximately two-thirds of the expected amount of sCD4-177 was recovered by OKT4 immunoprecipitation. We feel that the most likely explanation for this reduced recovery is dissociation of some of the Env dimers during the immunoprecipitation procedure, though other possibilities such as steric hindrance following a single binding event may also play a role.

We and others have shown that the Env proteins of HIV-2 and simian immunodeficiency virus (SIV) are also oligomeric (7, 8, 27, 28). Furthermore, they share a structurally and functionally conserved assembly domain with the HIV-1 Env protein (8). We infected cells with a recombinant vaccinia virus which expresses the SIV Env protein (16) to determine whether it too exhibits multimeric CD4 binding. As shown in Fig. 2B, the results obtained with SIV Env were essentially identical to those obtained with HIV-1 Env. Thus, it too exhibits multimeric CD4 binding. While we have not tested the ability of the HIV-2 Env protein to bind

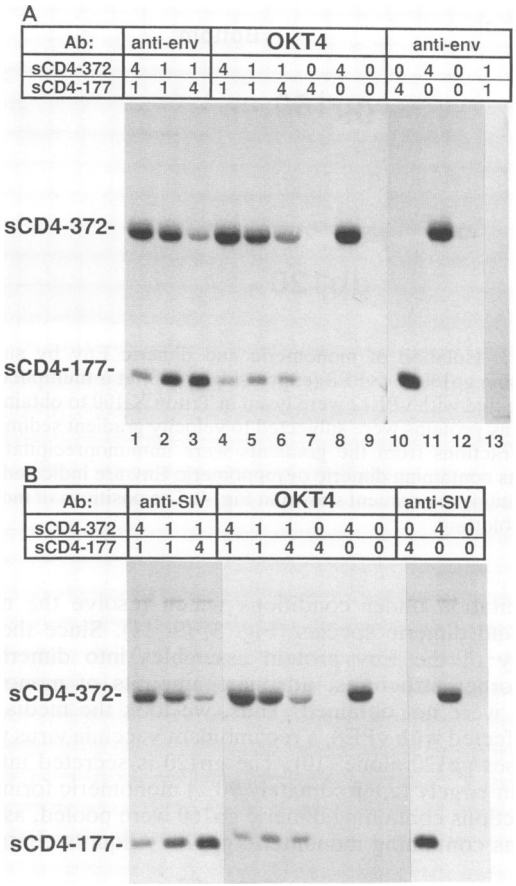


FIG. 2. Multimeric CD4 binding exhibited by cell surface HIV-1 and SIV Env proteins. To prepare radiolabeled sCD4, BSC1 cells were coinfected with vTF7-3 and either vEB4 (which expresses sCD4-177) or vTMEB10 (which expresses sCD4-372) at a multiplicity of 10 PFU per cell. Both vEB4 and vTMEB10 were made by standard procedures, using thymide kinase selection from plasmids ($pCD4_t$ and pCD4LTM1, respectively) made by E. A. Berger (3, 21). Cells were labeled with either [$^{35}$S]methionine (vTMEB10) or [$^{35}$S]cysteine (vEB4). Labeling conditions were adjusted empirically so as to obtain similar specific activities for both sCD4-372 and sCD4-177. At 24 h p.i., the media were harvested and concentrated twofold by centrifugation in Centricon 10 microconcentrators. Anisomycin was then added to a final concentration of 5 μg/ml to prevent labeling of Env during subsequent incubation of sCD4 with Env-infected cells. To prepare Env-infected cells, BSC1 cells were infected with either vPE12, which expresses a noncleavable form of HIV-1 Env (A), or WR194, which expresses SIV Env (B), using a multiplicity of 5 PFU per cell. These cells were not metabolically labeled so that rigorous washes during immunoprecipitation, which could disrupt Env-CD4 interactions, would not have to be performed. At 16 h p.i., the cells were washed with PBS and incubated at 37°C for 3 h with sCD4-177 and sCD4-372 in various concentrations. The relative amounts of sCD4-177 and sCD4-372 are shown. Cells were then washed twice with PBS to remove unbound sCD4 and lysed with Triton X-100. Each lysate was divided into two aliquots and immunoprecipitated with either OKT4 (lanes 4 to 9) or anti-Env antibody (Ab) (lanes 1 to 3 and 10 to 13), as indicated.

multiple CD4 molecules, we note that it is highly homologous to the SIV Env protein and thus is likely to do so.

To further test the ability of oligomeric Env protein to bind multiple CD4 molecules, we isolated monomeric and dimeric Env protein from sucrose velocity gradients. Cells expressing gp160 were lysed and subjected to velocity gradient

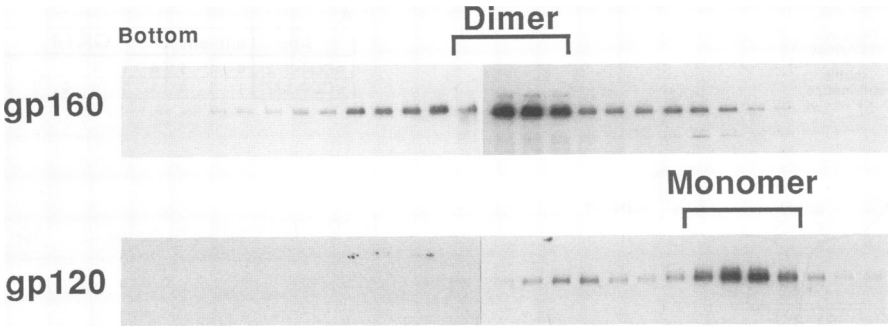


FIG. 3. Isolation of monomeric and dimeric Env by sucrose gradient centrifugation. BSC1 cells were infected with either vPE12 (expressing gp160) or vPE8 (expressing gp120) at a multiplicity of 30 PFU per cell. Cells were labeled overnight with [$^{35}$S]methionine. The cells infected with vPE12 were lysed in Triton X-100 to obtain gp160, while gp120 was obtained from the medium of cells infected with vPE8. These Env proteins were subjected to velocity gradient sedimentation on 5 to 20% sucrose gradients at 4°C for 20 h at 40,000 rpm in an SW40 rotor. Fractions from the gradients were immunoprecipitated with anti-Env antibody and analyzed on SDS–10% polyacrylamide gels. Fractions containing dimeric or monomeric Env are indicated. Parallel gradients with unlabeled Env were also run to obtain material for the sCD4 binding experiment shown in Fig. 4. The positions of monomeric and dimeric Env protein in these gradients were confirmed by Western immunoblotting.

sedimentation under conditions which resolve the monomeric and dimeric species (Fig. 3) (9, 11). Since the vast majority of the Env protein assembles into dimers and higher-order structures, adequate amounts of monomeric protein were not obtained. Thus, we took the media from cells infected with vPE8, a recombinant vaccinia virus which expresses gp120 alone (10). The gp120 is secreted into the media in largely (approximately 90%) monomeric form (Fig. 3). Fractions containing dimeric gp160 were pooled, as were fractions containing monomeric gp120. Aliquots containing approximately equivalent amounts of Env protein were then incubated for 3 h at 37°C with excess sCD4-177 and sCD4-372. The samples were then divided into two portions and immunoprecipitated with either anti-Env or OKT4 (Fig. 4). When monomeric or dimeric Env protein was immunoprecipitated with anti-Env, both sCD4-372 and sCD4-177 were coprecipitated. When OKT4 was used, both Env-bound and excess unbound sCD4-372 were precipitated regardless of whether monomeric or dimeric form of Env was used. However, sCD4-177 was coprecipitated only when dimeric Env protein was present. The amount of sCD4-177 coprecipitated by OKT4 was similar to the amount precipitated with anti-Env. sCD4-177 was not coprecipitated by OKT4 when incubated with monomeric Env and sCD4-372. Thus, monomeric Env protein binds only one CD4 molecule, while dimeric Env protein binds two. Because sCD4-372 was present in excess over Env, much larger amounts were precipitated with OKT4 than with anti-Env. This was not the case in the cell surface binding experiments (Fig. 2) in which sCD4 not bound to Env was removed prior to immunoprecipitation.

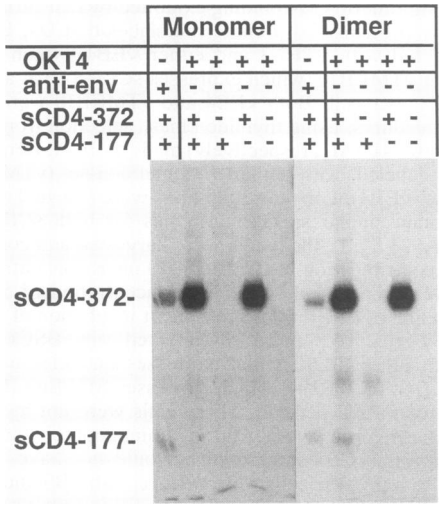


FIG. 4. Binding of sCD4 to purified monomeric and dimeric Env protein. BSC1 cells were infected with either vPE12 (noncleavable gp160) or vPE16 (cleavable gp160) at a multiplicity of 30 PFU per cell. For cells infected with vPE16, the virus inoculum was removed after 1 h and cells were overlayed with Optimem (GIBCO). At 24 h p.i., the medium (containing shed gp120) was harvested and concentrated in a Centricon 10 microconcentrator. Cells infected with vPE12 were lysed with Triton X-100 at 24 h p.i. Env protein was purified as described for Fig. 3. Fractions containing monomeric gp120 or dimeric gp160 were pooled. Equivalent amounts of monomeric or dimeric Env were incubated with excess metabolically labeled sCD4-372 and/or sCD4-177, as indicated, for 3 h at 37°C. Complexes were then immunoprecipitated with either OKT4 or anti-Env and analyzed on SDS–10% polyacrylamide gels.

Evidence for binding of two CD4 molecules also was obtained when secreted forms of oligomeric Env protein were used. The transmembrane and cytoplasmic domains of gp41 were eliminated by inserting a stop codon immediately before the transmembrane coding region. As a result, the ectodomain fragment, termed gp140, is secreted from cells (10a). Monomeric, dimeric, and tetrameric forms of gp140 were isolated by velocity gradient centrifugation. To detect multimeric binding, protein A-coated Sepharose beads were incubated with OKT4, washed, and incubated with metabolically labeled sCD4-372. Unbound sCD4 was removed by washing, after which monomeric, dimeric, or tetrameric Env protein was added. Unbound Env was removed, after which the immune complexes were incubated with metabolically labeled sCD4-177. After washing, the samples were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Fig. 5). Once again, sCD4-177 was coprecipitated by OKT4 only in the presence of oligomeric Env protein, confirming that Env protein dimers and tetramers display multimeric CD4 binding.

Most viral membrane proteins studied to date are oligomeric (14, 29). The Env proteins of HIV-1, HIV-2, and SIV form noncovalently associated dimers shortly after synthesis

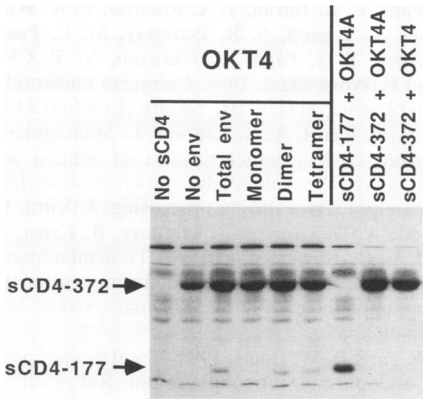


FIG. 5. Binding of sCD4 to secreted monomeric and oligomeric Env protein. Protein A-Sepharose beads (coated with rabbit anti-mouse antibody) were incubated with OKT4 antibody for 1 h, washed twice with Triton buffer (100 mM Tris-HCl [pH 8.0], 100 mM NaCl, 0.5% Triton X-100), then incubated with metabolically labeled sCD4-372 for 1 h, and washed twice with Triton buffer to remove unbound sCD4. Env protein was prepared from BSC1 cells infected with vPE12B, a recombinant vaccinia virus which produces a soluble secreted form of Env composed of the entire ectodomain and oligomerizes like the wild-type Env protein (10a). The immune complexes were then incubated for 45 min with different Env gradient fractions or Env that was not gradient purified, as indicated. After two washes with Triton buffer, metabolically labeled sCD4-177 was added and incubation continued for 45 min. Beads were then washed four times with Triton buffer and analyzed on SDS–10% polyacrylamide gels. Approximately one-half of the amount of sCD4-372 and sCD4-177 that was used for these reactions was immunoprecipitated with either OKT4 or OKT4A as a marker.

(7–9, 11, 27, 28). Assembly into higher-order forms, most likely a tetrameric structure composed of two dimers, is also likely to occur (9, 25, 33, 34, 36). Env protein oligomerization has several structural and functional implications. As with other oligomeric proteins (14, 29), mutations which prevent subunit folding and assembly are likely to prevent Env protein transport from the endoplasmic reticulum to the cell surface (37). Antigenic epitopes contingent upon the molecule's quaternary structure might also exist, as has been found for several other viral proteins (1, 31). An additional and obvious functional consequence of the env protein's oligomeric structure is that it has the potential to bind multiple CD4 molecules. Our results show that Env protein dimers, the predominant oligomeric form of the Env protein when expressed by recombinant vaccinia viruses, bind more than one CD4 molecule. Since monomeric Env protein binds one CD4 molecule, the dimer presumably binds two. This finding supports the hypotheses of Moore et al. (23, 24) and Layne et al. (18), who have proposed that multimeric CD4 binding by Env protein oligomers is required for gp120 dissociation as well as for viral adsorption and penetration. Thus, the conformational change undergone by the Env protein oligomer, which leads to membrane fusion, may be a cooperative event. We note that another viral membrane fusion protein, the influenza virus hemagglutinin trimer, also undergoes a highly cooperative conformational change (6).

While we have shown that Env protein dimers can bind two CD4 molecules, we do not yet know whether allosteric mechanisms are involved. A recent study found significant reductions in the binding of sCD4 to intact virions (which contain oligomeric Env protein) of five primary isolates in comparison with the binding seen with soluble (monomeric) gp120 isolated from the same viruses (22). It was suggested that the reduced binding was a consequence of the tertiary or quaternary structure of the Env protein on intact virions (22). If CD4 binding by individual gp120 subunits is in some way negatively modulated by the protein's oligomeric structure, then the intact virus may be rendered more resistant to neutralization by sCD4 and sCD4-immunoglobulin G chimeras (22). We are currently attempting to obtain quantities of secreted monomeric and dimeric forms of Env protein sufficient to allow us to determine their affinities for CD4. Such studies should further clarify the functional significance and clinical implications of Env protein oligomerization.

We thank Ed Berger for providing the recombinant vaccinia viruses which express sCD4-177 and sCD4-372 and Ron Willey and Bruce Chesbro for anti-Env antibodies. We also thank Norman Cooper for providing cells and viruses.

## REFERENCES

1. **Ada, G. L.** 1991. Vaccination and the immune response. Curr. Biol. **1**:221–223.
2. **Allan, J. S., J. Strauss, and D. W. Buck.** 1990. Enhancement of SIV infection with soluble receptor molecules. Science **247**: 1084–1088.
3. **Berger, E. A., T. R. Fuerst, and B. Moss.** 1988. A soluble recombinant polypeptide comprising the amino-terminal half of the extracellular region of the CD4 molecule contains an active binding site for human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **85**:2357–2361.
4. **Berger, E. A., J. D. Lifson, and L. E. Eiden.** 1991. Stimulation of glycoprotein gp120 dissociation from the envelope glycoprotein complex of human immunodeficiency virus type 1 by soluble CD4 and CD4 peptide derivatives: implications for the role of the complementarity-determining region 3-like region in membrane fusion. Proc. Natl. Acad. Sci. USA **88**:8082–8086.
5. **Bosch, M. L., P. L. Earl, K. Fargnoli, S. Picciafuoco, F. Giombini, F. Wong-Staal, and G. Franchini.** 1989. Identification of the fusion peptide of primate immunodeficiency viruses. Science **244**:694–697.
6. **Boulay, F., R. W. Doms, R. G. Webster, and A. Helenius.** 1988. Posttranslational oligomerization and cooperative acid activation of mixed influenza hemagglutinin trimers. J. Cell Biol. **106**:629–639.
7. **Chakrabarti, S., T. Mizukami, G. Franchini, and B. Moss.** 1990. Synthesis, oligomerization, and biological activity of the human immunodeficiency virus type 2 envelope glycoprotein expressed by a recombinant vaccinia virus. Virology **178**:134–142.
8. **Doms, R. W., P. L. Earl, S. Chakrabarti, and B. Moss.** 1990. The HIV-1, HIV-2, and SIV envelope glycoproteins share a functionally conserved assembly domain. J. Virol. **64**:3537–3540.
9. **Earl, P. L., R. W. Doms, and B. Moss.** 1990. Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein. Proc. Natl. Acad. Sci. USA **87**:648–652.
10. **Earl, P. L., S. Koenig, and B. Moss.** 1991. Biological and immunological properties of the HIV-1 envelope glycoprotein: analysis of proteins with truncations and deletions expressed by recombinant vaccinia viruses. J. Virol. **65**:31–41.

10a. **Earl, P. L., and B. Moss.** Unpublished data.

11. **Earl, P. L., B. Moss, and R. W. Doms.** 1991. Folding, interaction with GRP78-BiP, assembly, and transport of the human immunodeficiency virus type 1 envelope protein. J. Virol. **65**:2047–2055.
12. **Freed, E. O., D. J. Myers, and R. Risser.** 1990. Characterization of the fusion domain of the human immunodeficiency virus type 1 envelope glycoprotein gp41. Proc. Natl. Acad. Sci. USA **87**:4650–4654.
13. **Hart, T. S., R. Kirsh, H. Ellens, R. W. Sweet, D. M. Lambert, S. R. Petteway, J. Leary, and P. J. Bugelski.** 1991. Binding of soluble CD4 proteins to human immunodeficiency virus type 1 and infected cells induces release of envelope glycoprotein gp120. Proc. Natl. Acad. Sci. USA **88**:2189–2193.

14. **Hurtley, S. M., and A. Helenius.** 1989. Protein oligomerization in the endoplasmic reticulum. Annu. Rev. Cell. Biol. **5**:277–307.
15. **Kirsh, R., T. K. Hart, H. Ellens, J. Miller, S. A. Petteway, Jr., D. M. Lambert, J. Leary, and P. J. Bugelski.** 1990. Morphometric analysis of recombinant soluble CD4-mediated release of the envelope glycoprotein gp120 from HIV-1. AIDS Res. Hum. Retroviruses **6**:1209–1212.
16. **Koenig, S., V. M. Hirsch, R. A. Olmstead, D. Powell, W. Maury, A. Rabson, A. S. Fauci, R. H. Purcell, and P. R. Johnson.** 1989. Selective infection of human $CD4^+$ cells by simian immunodeficiency virus: productive infection associated with envelope glycoprotein-induced fusion. Proc. Natl. Acad. Sci. USA **86**: 2443–2447.
17. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwillger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the envelope glycoprotein of human immunodeficiency virus type I. Science **237**:1351–1355.
18. **Layne, S. P., M. J. Merges, M. B. Dembo, J. L. Spouge, and P. L. Nara.** 1990. HIV requires multiple gp120 molecules for CD4-mediated infection. Nature (London) **346**:277–279.
19. **McCune, J. M., L. B. Rabin, M. B. Feinberg, M. Lieberman, J. C. Kosek, G. R. Reyes, and I. L. Weissman.** 1988. Endoproteolytic cleavage of gp160 is required for the activation of human immunodeficiency virus. Cell **53**:55–67.
20. **McKeating, J. A., and R. L. Willey.** 1989. Structure and function of the HIV envelope. AIDS **3**:S35–S41.
21. **Mizukami, T., T. R. Fuerst, E. A. Berger, and B. Moss.** 1988. Binding region for human immunodeficiency virus (HIV) and epitopes for HIV-blocking antibodies of the CD4 molecule defined by site-directed mutagenesis. Proc. Natl. Acad. Sci. USA **85**:9273–9277.
22. **Moore, J. P., J. A. McKeating, Y. Huang, A. Ashkenazi, and D. D. Ho.** 1992. Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. J. Virol. **66**:235–243.
23. **Moore, J. P., J. A. McKeating, W. A. Norton, and Q. J. Sattentau.** 1991. Direct measurement of soluble CD4 binding to human immunodeficiency virus type 1 virions: gp120 dissociation and its implications for virus-cell binding and fusion reactions and their neutralization by soluble CD4. J. Virol. **65**:1133–1140.
24. **Moore, J. P., J. A. McKeating, R. A. Weiss, and Q. J. Sattentau.** 1990. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. Science **250**:1130–1142.
25. **Pinter, A., W. J. Honnen, S. A. Tilley, C. Bona, H. Zaghouani, M. K. Gorny, and S. Zolla-Pazner.** 1989. Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. J. Virol. **63**:2674–2679.
26. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1984. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313**:277–284.
27. **Rey, M.-A., B. Krust, A. G. Laurent, L. Montagnier, and A. G. Hovanessian.** 1989. Characterization of human immunodeficiency virus type 2 envelope glycoproteins: dimerization of the glycoprotein precursor during processing. J. Virol. **63**:647–658.
28. **Rey, M.-A., A. G. Laurent, J. McClure, B. Krust, L. Montagnier, and A. G. Hovanessian.** 1990. Transmembrane envelope glycoproteins of human immunodeficiency virus type 2 and simian immunodeficiency virus SIV-mac exist as homodimers. J. Virol. **64**:922–926.
29. **Rose, J. K., and R. W. Doms.** 1988. Regulation of protein export from the endoplasmic reticulum. Annu. Rev. Cell Biol. **4**:257–288.
30. **Ryu, S.-E., P. D. Kwong, A. Truneh, T. G. Porter, J. Arthos, M. Rosenberg, X. Dai, N. Xuong, R. Axel, R. W. Sweet, and W. A. Hendrickson.** 1991. Crystal structure of an HIV-binding recombinant fragment of human CD4. Nature (London) **348**:419–426.
31. **Sanjuan, A., Y. Quesneau, J. F. Delagneau, and F. Brun-Vezinet.** 1991. Value of HIV transmembrane glycoprotein oligomers as target antigens in early detection of anti-env antibodies. AIDS Res. Hum. Retroviruses **7**:789–790.
32. **Sattentau, Q. J., and J. P. Moore.** 1991. Conformational changes in the human immunodeficiency virus envelope glycoproteins by soluble CD4 binding. J. Exp. Med. **174**:407–415.
33. **Schawaller, M., C. E. Smith, J. J. Skehel, and D. C. Wiley.** 1989. Studies with cross-linking reagents on the oligomeric structure of the env-glycoprotein of HIV. Virology **172**:367–369.
34. **Thomas, D. J., J. S. Wall, J. F. Hainfeld, M. Kaczorek, F. P. Booy, B. L. Trus, F. A. Eiserling, and A. C. Steven.** 1991. gp160, the envelope glycoprotein of human immunodeficiency virus type 1, is a dimer of 125-kilodalton subunits stabilized though interactions between their gp41 domains. J. Virol. **65**:3797–3803.
35. **Wang, J., Y. Yan, T. P. J. Garrett, J. Liu, D. W. Rodgers, R. L. Garlick, G. E. Tarr, Y. Husain, E. L. Reinherz, and S. C. Harrison.** 1990. Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. Nature (London) **348**:411–418.
36. **Weiss, C. D., J. A. Levy, and J. M. White.** 1990. Oligomeric organization of gp120 on infectious immunodeficiency virus type 1 particles. J. Virol. **64**:5674–5677.
37. **Willey, R. L., T. Klimkait, D. M. Frucht, J. S. Bonifacino, and M. A. Martin.** 1991. Mutations within the human immunodeficiency virus type 1 gp160 envelope glycoprotein alter its intracellular transport and processing. Virology **184**:319–329.