gp120-Independent Fusion Mediated by the Human Immunodeficiency Virus Type 1 gp41 Envelope Glycoprotein: a Reassessment

LUISA MARCON¹ AND JOSEPH SODROSKI^{1,2*}

Division of Human Retrovirology, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School,¹ and Department of Cancer Biology, Harvard School of Public Health,² Boston, Massachusetts 02115

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In a natural context, membrane fusion mediated by the human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins involves both the exterior envelope glycoprotein (gp120) and the transmembrane glycoprotein (gp41). Perez et al. (J. Virol. 66:4134–4143, 1992) reported that a mutant HIV-1 envelope glycoprotein containing only the signal peptide and carboxyl terminus of the gp120 exterior glycoprotein fused to the complete gp41 glycoprotein was properly cleaved and that the resultant gp41 glycoprotein was able to induce the fusion of even CD4-negative cells. In the studies reported herein, mutant proteins identical or similar to those studied by Perez et al. lacked detectable cell fusion activity. The proteolytic processing of these proteins was very inefficient, and one processed product identified by Perez et al. as the authentic gp41 glycoprotein was shown to contain carboxyl-terminal gp120 sequences. Furthermore, no fusion activity was observed for gp41 glycoproteins exposed after shedding of the gp120 glycoprotein by soluble CD4. Thus, evidence supporting a gp120-independent cell fusion activity for the HIV-1 gp41 glycoprotein is currently lacking.

The envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS, mediate virus entry and cytopathic effects (2, 29, 38, 52, 55, 57). The gp160 envelope glycoprotein precursor is synthesized in the endoplasmic reticulum and is subsequently cleaved in a late Golgi compartment to yield the exterior envelope glycoprotein (gp120) and the transmembrane glycoprotein (gp41) (17, 18, 58, 62, 65). Recently furin, a subtilisin-like endoprotease, has been shown to cleave the HIV-1 gp160 glycoprotein in HeLa cells and in T-lymphocytic cell lines (27). Following cleavage, the gp120 glycoprotein remains anchored to the cellular or viral membrane through noncovalent bonds to the gp41 glycoprotein (30, 34a, 36).

HIV-1 entry and cytopathic effects involve the envelope glycoprotein-mediated fusion of virus-cell and cell-cell membranes, respectively. The initial event in these processes is the binding of the gp120 glycoprotein to the CD4 receptor (13, 35, 39, 41, 42). The CD4 binding region of gp120 consists of discontinuous sequences located in the third and fourth conserved regions (11, 12, 37, 46). Mutagenic and neutralizing-antibody studies have suggested that specific regions of both the gp120 and gp41 glycoproteins are important for post-receptor binding steps involved in membrane fusion. Amino acid changes in the second (V2) and third (V3) variable loops of the gp120 glycoprotein, which serve as targets for neutralizing antibodies, as well as in the fourth conserved (C4) region of the gp120 glycoprotein, can decrease fusion potential without disrupting receptor binding (7, 21, 26, 34, 47, 59–61).

Mutagenic studies have also defined functional regions on the gp41 transmembrane glycoprotein, which contains an external domain, a transmembrane region, and an intracyto-

plasmic tail. The external domain is involved in oligomerization, association with the gp120 glycoprotein, and membrane fusion (10, 15, 16, 20, 36). The transmembrane domain mainly serves to anchor the viral glycoproteins in the viral or cell membranes, although some alterations in the transmembrane region can affect the fusion function (22, 31). The intracytoplasmic gp41 domain plays a role in HIV-1 entry other than receptor binding or fusion (14, 23).

Among the gp41 regions implicated in fusion activity, a discrete stretch of hydrophobic amino acids, referred to as the fusion peptide, is located at the amino terminus of the glycoprotein (24). Proper cleavage of the gp160 glycoprotein precursor frees the gp41 fusion peptide from gp120 sequences and is a prerequisite for fusion functions (40). Genetic evidence supports the hypothesis that the HIV-1 gp41 fusion peptide interacts with the target cell during the membrane fusion process (7). The concept that gp41 exposure to the target cell is involved in membrane fusion suggests that conformational changes in the envelope glycoproteins are likely to be necessary to effect exposure of gp41 regions buried on the native glycoprotein complex. Such conformational changes could be triggered by CD4 binding. It has been observed that a soluble form of CD4 induces shedding of the HIV-1 gp120 glycoprotein (3, 5, 9, 28, 43, 44, 53), leading to exposure of previously hidden gp41 epitopes (56). However, more recent studies indicate a lack of correlation between the fusion capacity and gp120-shedding properties of a panel of HIV-1 envelope glycoprotein mutants (6, 60). Therefore, a simple model in which receptor binding removes the gp120 exterior envelope glycoprotein from the functional fusion apparatus appears inadequate to explain HIV-1 envelope-mediated membrane fusion.

It has been reported that deletion of the majority of the gp120 sequences from the HIV-1_{BRU} envelope glycoprotein resulted in a truncated precursor molecule that was properly cleaved to yield the gp41 glycoprotein (49). It was also reported that this gp41 glycoprotein mediated cell-cell fusion

* Corresponding author. Mailing address: Division of Human Retrovirology, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115. Phone: (617) 632-3371. Fax: (617) 632-4338. Electronic mail address: Joseph_Sodroski@DFCI.Harvard.edu.

FIG. 1. Schematic representation for the construction of the plasmid expressing the gp41 envelope glycoprotein. A plasmid expressing the wild-type envelope glycoproteins (pSVIIIenv) was first digested with the restriction enzymes *Kpn*I and *Bgl*II and, after mung bean nuclease digestion, religated. The resulting plasmid encodes a protein containing a deletion between amino acids 41 and 468; the amino acid sequence at the junction is shown. S, signal peptide; K, *Kpn*I; B, *Bgl*II. Numbers indicate the nucleotides of the HXBc2 strain of HIV-1.

in both CD4-positive and CD4-negative cells. Here we reexamine the structural and functional properties of glycoprotein products derived from similar or identical constructs.

Expression of a deleted HIV-1 envelope glycoprotein. A construct designed to express an HIV-1 envelope glycoprotein with a deletion in the gp120 moiety analogous to that described by Perez et al. (49) was made, by using an *env* gene derived from the HXBc2 strain of HIV-1 (55). After cleavage with *Kpn*I and *Bgl*II, blunt ends were generated with mung bean nuclease prior to ligation and *Escherichia coli* transformation (Fig. 1). The resultant plasmid, pSVIIIenvΔ(42-467), should encode a protein containing the signal peptide and the adjacent 11 amino acids of the gp120 glycoprotein, the carboxyl-terminal 44 amino acids of the gp120 glycoprotein, and the entire gp41 sequence. A negative control plasmid containing a frameshift mutation at the site of the deletion (pSVIIIFS) was also generated. Plasmids designed to express the wild-type and the deletion-containing envelope glycoproteins (referred to herein as deleted glycoproteins) under the control of the cytomegalovirus immediate-early promoter were also constructed [pCMVenv and pCMVenvΔ(42-467)]. All the plasmids were sequenced to ensure that the *env* reading frame was preserved at the site of the deletion.

COS-1 cells were transfected by the DEAE-dextran method (12a) with the plasmids expressing either the wild-type or the deleted envelope glycoproteins and with the control plasmid containing the frameshift mutation. At 48 h after transfection, cells were labeled overnight with [³⁵S]cysteine and [³⁵S]methionine. The cells were then lysed in buffer containing 500 mM NaCl, 10 mM Tris [pH 7.5], and 0.5% Nonidet P-40. Cell lysates were precipitated with anti-gp41 monoclonal antibodies 50-69 and 98-6 (25, 50, 67). The monoclonal antibody 50-69 recognized both gp160 and gp41 glycoproteins in the lysates of cells transfected with the plasmid expressing the wild-type envelope glycoproteins (Fig. 2, lane 2). The 50-69 antibody precipitated a 50- to 52-kDa protein from cells transfected with the pSVIIIenvΔ(42-467) plasmid (Fig. 2, lane 3). This corresponds to the size expected for the uncleaved precursor form of the deleted glycoprotein. Smaller amounts of proteins migrating at approximately 39 kDa and at approximately 110 kDa were also precipitated by the anti-gp41 monoclonal antibody. On some gels, a trace amount of a 37-kDa species

FIG. 2. Expression of wild-type and gp120-deleted envelope glycoproteins in COS-1 cells. Results of radioimmunoprecipitation of cell lysates transfected with pSVIIIFS (lane 1), pSVIIIenv (lane 2), pSVIIIenvΔ(42-467) (lane 3), pwtENV (lane 4), or ptrENV (lane 5) with monoclonal antibody 50-69, which recognizes a region of gp41 between amino acids 599 and 613, are shown.

could be detected (Fig. 3). Similar results were obtained with the 98-6 anti-gp41 monoclonal antibody (data not shown). Proteins migrating on the sodium dodecyl sulfate (SDS)-polyacrylamide gels identically to those synthesized by the pSVIIIenvΔ(42-467) plasmid were precipitated by the anti-gp41 monoclonal antibody 50-69 from COS-1 cells transfected with the ptrENV plasmid, obtained from Lautaro Perez (Fig. 2, lanes 3 and 5). This plasmid expresses a deleted envelope glycoprotein derived from HIV-1_{BRU}. None of the proteins were precipitated from cells transfected with the pSVIIIFS plasmid.

The 39- and 37-kDa proteins presumably represent proteo-

FIG. 3. Expression and processing of wild-type and gp120-deleted envelope glycoproteins in COS-1 cells. Results of radioimmunoprecipitation of cell lysates transfected with pCMV control (lanes 1 and 4), pCMVIIenv (lanes 2 and 5), and pCMVenvΔ(42-467) (lanes 3 and 6) with antibody 50-69 (lanes 1 through 3) and a rabbit polyclonal antibody raised against peptide C24A (amino acids 489 to 511) (lanes 4 through 6). Slight differences in the migration of the pCMVenvΔ(42-467) precursor such as those observed between lanes 3 and 6 were not detectable in several other independent experiments. Arrows indicate the cleaved species of 39 and 37 kDa.

TABLE 1. Syncytium formation assays

Transfecting plasmid	CD4 ⁺ HeLa cell transfection			No. of syncytia/10 HPF ^a after COS-1 transfection and SupT1 cocultivation
	No. of adherent blue syncytia/10 HPF	No. of nuclei/syncytium ^b	No. of syncytia in cell supernatants/10 HPF	
pSVIIIFS	0	NA	0	0
pSVIIEnv	20	13	80–100	20–60
pSVIIEnvΔ(42–467)	0	NA	0	0

^a HPF, high-power fields.^b NA, not applicable.

lytic cleavage products derived from the 50- to 52-kDa glycoprotein precursor. The 110-kDa species possibly represents an oligomer or aggregate derived from either the glycoprotein precursor or cleaved products. Attempts to disrupt the 110-kDa species by boiling in the presence of SDS of up to 4% were unsuccessful (data not shown). We thus cannot distinguish whether this species represents SDS-resistant oligomers, which have been described for HIV-1, HIV-2, and simian immunodeficiency virus, or abnormal aggregates (8, 16, 50, 51, 54).

Syncytium-forming ability of deleted glycoproteins. The ability of the deleted glycoprotein encoded by the pSVIIEnvΔ(42–467) plasmid to induce cell-cell fusion was examined in two assays. In the first assay, CD4⁺ HeLa cells (source, R. Axel) were cotransfected with 10 μg each of pSVIIIFS, pSVIIEnv, and pSVIIEnvΔ(42–467) DNA together with 10 μg of a plasmid encoding β-galactosidase under the control of the HIV-1 long terminal repeat (50a). At 60 h after transfection, cells were fixed with 3% paraformaldehyde and then stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Supernatants from the transfected cells were also collected and scored for the presence of syncytia that had detached from the surface of the tissue culture dish.

Cells transfected with the pSVIIEnv and β-galactosidase-expressing plasmids demonstrated an average of 20 blue syncytia per 10 high-power fields, with each syncytium containing an average of 13 nuclei (Table 1). Eighty to 100 syncytia per 10 high-power fields were seen in the supernatants collected from these cells. By contrast, no syncytia were observed in either the supernatants or the plates transfected with either the pSVIIEnvΔ(42–467) or the pSVIIIFS plasmid.

A second syncytium assay in which COS-1 cells transfected with the pSVIIIFS, pSVIIEnv, and pSVIIEnvΔ(42–467) plasmids were cocultivated with SupT1 CD4-positive lymphocytes were performed. No syncytia were observed in any of the transfected COS-1 cultures prior to cocultivation with SupT1 cells. The COS-1 cells transfected with the pSVIIEnv plasmid formed 20 to 60 syncytia per 10 high-power fields upon cocultivation with SupT1 cells. By contrast, there were no syncytia observed after SupT1 cocultivation with COS-1 cells transfected with either the pSVIIIFS or the pSVIIEnvΔ(42–467) plasmid (Table 1).

The ptrENV plasmid, obtained from Lautaro Perez, was also tested in both syncytium formation assays. No syncytia were induced by this plasmid in either assay (data not shown). We also examined whether the proteins encoded by the pSVIIEnvΔ(42–467) and pCMVenvΔ(42–467) plasmids could exert a dominant negative effect on syncytium formation mediated by the wild-type HIV-1 envelope glycoproteins. Even when the molar expression of the deleted envelope glycoproteins was twice as great as that of the wild-type glycoproteins, no significant inhibitory effect was seen (data not shown).

Processing of the deleted envelope glycoproteins. The lack of detectable cell fusion activity by the deleted envelope glyco-

proteins prompted a further characterization of these proteins. Radiolabeled lysates from COS-1 cells transfected with pSVIIIFS, pSVIIEnv, or pSVIIEnvΔ(42–467) plasmids were precipitated either with the 50-69 monoclonal antibody, which recognizes the gp41 ectodomain, or a polyclonal rabbit anti-serum raised against a peptide corresponding to the carboxyl-terminal (C5) portion of the gp120 glycoprotein (4). Labeling and immunoprecipitation were carried out as described above.

Figure 3 demonstrates that, as expected, the anti-C5 serum precipitated both the gp160 and the gp120 glycoproteins, but not the gp41 glycoprotein, from lysates derived from cells transfected with the pSVIIEnv plasmid (lane 5). The rabbit anti-C5 also recognized the 50- to 52-kDa precursor protein encoded by the pSVIIEnvΔ(42–467) plasmid, as expected (Fig. 3, lane 6). Unexpectedly, the rabbit anti-C5 serum precipitated both the 39-kDa protein and a less abundant 37-kDa species from pSVIIEnvΔ(42–467)-transfected cell lysates (Fig. 3, lane 6). A 42-kDa background band was also precipitated by the anti-C5 serum. Identical results were obtained with the ptrENV plasmid, obtained from Lautaro Perez (data not shown). These results indicate that the deleted precursor molecules encoded by these plasmids are not properly cleaved at the authentic gp120-gp41 cleavage site, since all gp41-related products also contain gp120 sequences.

Testing the cell-fusing ability of gp41 after gp120 shedding.

Experiments were designed to analyze whether the wild-type gp41 glycoprotein, after removal of the gp120 glycoprotein, would be able to mediate fusion with SupT1 target lymphocytes. Soluble CD4 has been shown to induce the shedding of the HIV-1 gp120 glycoprotein from the cell membrane envelope glycoprotein complex (28, 43). Treatment of cells expressing the HIV-1 envelope glycoproteins with soluble CD4 has been demonstrated to expose gp41 epitopes presumably masked by the gp120 glycoprotein (53a, 56). Evaluating the fusogenic potential of the soluble-CD4-exposed gp41 glycoprotein by using the wild-type HIV-1 envelope glycoproteins is complicated by the cell-fusing ability of the nondissociated envelope glycoprotein complexes. Instead, mutant HIV-1 envelope glycoproteins, ΔV3 and 314 G/Q, containing a deletion and a substitution, respectively, in the third variable (V3) region of the gp120 moiety were used (60, 66). These mutants are properly processed, are expressed on the cell surface, and efficiently shed the gp120 glycoprotein in response to soluble CD4 (60, 66), yet they are fusion defective. COS-1 cells were transfected with plasmids expressing the wild-type, 314 G/Q, and ΔV3 envelope glycoproteins or with control plasmids that do not express HIV-1 envelope glycoproteins. At 60 h following transfection, cells were washed in phosphate-buffered saline and treated with 50 μg of soluble CD4 per ml for 90 min at 37°C. Cells were then washed and cocultivated with SupT1 CD4-positive lymphocytes. Syncytium formation mediated by the wild-type HIV-1 envelope glycoproteins demonstrated a decrease associated with soluble-CD4 treatment (Table 2).

TABLE 2. Syncytium formation in the absence and presence of soluble CD4

Plasmid	No. of syncytia/24-well plate	
	Without soluble CD4	With soluble CD4
pSVIIIIFS	0	0
pSVIIIenv	239	70
pSVIIIΔV3	4	1-1
pSVIII314 G/Q	4	1-2
pSVIIIenvΔ(42-467)	0	0

The residual syncytium formation after soluble-CD4 treatment probably represents activity associated with envelope glycoproteins synthesized after soluble-CD4 treatment and newly expressed during the COS-1-SupT1 cell cocultivation. Neither the ΔV3- or 314 G/Q-expressing COS-1 cells exhibited efficient syncytium-forming ability, as previously reported. No increase in the number of syncytia formed was observed following soluble-CD4 treatment for either the ΔV3 or the 314 G/Q mutants.

A recent article by Perez et al. (49) reported that a molecule containing a deletion of most of the HIV-1 gp120 glycoprotein was properly processed to a mature, functional gp41 glycoprotein. This was unexpected in light of the phenotypes observed for mutant HIV-1 envelope glycoproteins containing gp120 amino acid changes resulting in gp120-gp41 dissociation after cleavage. In the case of every such mutant, the HIV-1 gp41 glycoprotein, although wild type in sequence, was rapidly turned over in intracellular compartments (10, 30, 34a, 36). These results suggested that the HIV-1 gp41 glycoprotein, in the absence of the gp120 exterior glycoprotein, may not readily retain native structure, a prerequisite for envelope glycoprotein transport to the cell surface. Thus, we attempted to verify the results of Perez et al. (49), using plasmids based on the HIV-1_{BRU} strain obtained directly from those investigators as well as analogous constructs made in our laboratory by using the HXBc2 strain of HIV-1. Identical results were obtained with both sets of constructs. The vast majority of the synthesized molecules in cells transfected with these plasmids represent unprocessed precursor glycoproteins of 50 to 52 kDa. A small fraction of the envelope glycoproteins was observed in the form of faster-migrating species of 39 and 37 kDa. Perez et al. (49) identified these faster-migrating species as the authentic gp41 glycoprotein. Our ability to precipitate these products with antiserum specific for the gp120 carboxyl terminus rules out the possibility that these represent mature gp41 glycoproteins. Since the exposure of the HIV-1 gp41 amino terminus is likely to be essential for membrane fusion, the ability of such improperly cleaved molecules to mediate fusion would be very unexpected. Indeed, in neither of the syncytium formation assays employed in this study could we detect any cell-fusing ability for either set of constructs designed to express gp120-deleted HIV-1 envelope glycoproteins.

We also examined whether the wild-type HIV-1 gp41 glycoprotein exposed by soluble-CD4-induced gp120 shedding might exhibit cell fusion activity. Using conditions previously demonstrated to expose masked gp41 epitopes on the cell surface, we did not observe any cell fusion attributable to the gp41 glycoprotein. These results are consistent with those of another study demonstrating that HIV-1 virions stripped of the gp120 glycoprotein by soluble CD4 were irreversibly inactivated for the ability to enter target cells (63). These negative results cannot conclusively prove that the HIV-1 gp41 glyco-

protein is incapable of mediating fusion in the absence of the gp120 exterior glycoprotein. Nonetheless, in conjunction with our inability to reproduce the findings of Perez et al. (49), the results indicate that evidence for a gp120-independent membrane fusion activity for the gp41 glycoprotein is currently lacking. That amino acid changes in multiple gp120 and gp41 regions affect post-receptor binding events involved in virus entry or cell fusion favors a model in which both HIV-1 envelope glycoproteins are essential for these processes.

For some enveloped viruses, glycoproteins implicated in membrane fusion are encoded by a gene separate from that which encodes the receptor-binding glycoprotein. For example, paramyxovirus fusion proteins expressed independently of other viral glycoproteins are properly processed and transported to the cell surface. The functional activity exhibited by these fusion proteins is typically lower than that observed when a full complement of envelope glycoproteins is present. In some cases, no cell fusion activity was observed unless the homologous receptor-binding glycoprotein was coexpressed with the fusion protein (1, 19, 32, 33, 45, 48, 64). Thus, even for viral envelope glycoproteins naturally expressed, processed, and transported as independent units, specific interprotein interactions appear to promote efficient function. Such interactions are likely to be even more critical for the function of envelope glycoproteins, like those of HIV-1, that are processed from a single precursor and exist as stable heterodimeric complexes.

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REFERENCES

1. Alkhatib, G., C. Richardson, and S.-H. Shen. 1990. Intracellular processing, glycosylation, and cell-surface expression of the measles virus fusion protein (F) encoded by a recombinant adenovirus. *Virology* 175:262-270.
2. Allan, J., T. H. Lee, M. F. McLane, J. Sodroski, W. A. Haseltine, and M. Essex. 1985. Identification of the major envelope glycoprotein product of HTLV-III. *Science* 228:1091-1094.
3. Allan, J., J. Strauss, and D. Buck. 1990. Enhancement of SIV infection with soluble receptor molecules. *Science* 247:1084-1088.
4. Ardman, B., M. Kowalski, J. Bristol, W. Haseltine, and J. Sodroski. 1990. Effects on CD4 binding of anti-peptide sera to the fourth and fifth conserved domains of HIV-1 gp120. *J. Acquired Immune Defic. Syndr.* 3:206-214.
5. Berger, E., J. Lifson, and L. 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.
6. Berger, E. A., J. R. Sisler, and P. L. Earl. 1992. Human immunodeficiency virus type 1 envelope glycoprotein molecules containing membrane fusion-impairing mutations in the V3 region efficiently undergo soluble CD4-stimulated gp120 release. *J. Virol.* 66:6208-6212.
7. Bergeron, L., N. Sullivan, and J. Sodroski. 1992. Target cell-specific determinants of membrane fusion within the human immunodeficiency virus type 1 gp120 third variable region and gp41 amino terminus. *J. Virol.* 66:2389-2397.
8. Berman, P. W., L. Riddle, G. Nakamura, O. K. Haffar, W. M. Nunes, P. Skehel, R. Byrn, J. Groopman, T. Matthews, and T.

- Gregory.** 1989. Expression and immunogenicity of the extracellular domain of the human immunodeficiency virus type 1 envelope glycoprotein, gp160. *J. Virol.* **63**:3489–3498.
9. **Bugelski, P., H. Ellens, T. Hart, and R. Kirsh.** 1991. Soluble CD4 and dextran sulfate mediate release of gp120 from HIV-1: implications for clinical trials. *J. Acquired Immune Defic. Syndr.* **4**:923–924.
 10. **Cao, J., L. Bergeron, E. Helseth, M. Thali, H. Repke, and J. Sodroski.** 1993. Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein. *J. Virol.* **67**:2747–2755.
 11. **Cordonnier, A., L. Montagnier, and M. Emerman.** 1989. Single amino acid changes in HIV envelope affect viral tropism and receptor binding. *Nature (London)* **340**:571–574.
 12. **Cordonnier, A., Y. Riviere, L. Montagnier, and M. Emerman.** 1989. Effects of mutations in hyperconserved regions of the extracellular glycoprotein of human immunodeficiency virus type 1 on receptor binding. *J. Virol.* **63**:4464–4468.
 - 12a. **Cullen, B. R.** 1987. Use of eukaryotic expression technology in the functional analysis of clone genes. *Methods Enzymol.* **152**:684–703.
 13. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature (London)* **312**:763–767.
 14. **Dubay, J. W., S. J. Roberts, B. H. Hahn, and E. Hunter.** 1992. Truncation of the human immunodeficiency virus type 1 transmembrane glycoprotein cytoplasmic domain blocks virus infectivity. *J. Virol.* **66**:6616–6625.
 15. **Dubay, J. W., S. J. Roberts, B. Brody, and E. Hunter.** 1992. Mutations in the leucine zipper of the human immunodeficiency virus type 1 transmembrane glycoprotein affect fusion and infectivity. *J. Virol.* **66**:4748–4756.
 16. **Earl, P., R. Doms, and B. Moss.** 1990. Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein. *Proc. Natl. Acad. Sci. USA* **87**:648–652.
 17. **Earl, P. L., S. Koenig, and B. Moss.** 1991. Biological and immunological properties of human immunodeficiency virus type 1 envelope glycoprotein: analysis of proteins with truncations and deletions expressed by recombinant vaccinia viruses. *J. Virol.* **65**:31–41.
 18. **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.
 19. **Ebata, S. N., M.-J. Côté, C. Yong Kang, and K. Dimock.** 1991. The fusion and hemagglutinin-neuraminidase glycoproteins of human parainfluenza virus 3 are both required for fusion. *Virology* **183**:437–441.
 20. **Freed, E., D. 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.
 21. **Freed, E. O., D. J. Myers, and R. Risser.** 1991. Identification of the principal neutralizing determinant of human immunodeficiency virus type 1 as a fusion domain. *J. Virol.* **65**:190–194.
 22. **Gabuzda, D., U. Olshevsky, P. Bertani, W. Haseltine, and J. Sodroski.** 1991. Identification of membrane anchorage domains of the HIV-1 gp160 envelope glycoproteins precursor. *J. Acquired Immune Defic. Syndr.* **4**:34–40.
 23. **Gabuzda, D. H., A. Lever, E. Terwilliger, and J. Sodroski.** 1992. Effects of deletions in the cytoplasmic domain on biological functions of the human immunodeficiency virus type 1 envelope glycoproteins. *J. Virol.* **66**:3306–3315.
 24. **Gallaher, W.** 1987. Detection of a fusion peptide sequence in the transmembrane protein of human immunodeficiency virus. *Cell* **50**:327–328.
 25. **Gorny, M. K., V. Gianakakos, S. Sharpe, and S. Zolla-Pazner.** 1989. Generation of human monoclonal antibodies to human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **86**:1624–1628.
 26. **Grimaila, R., B. Fuller, P. Rennert, M. B. Nelson, M.-L. Hammar-skjold, B. Potts, M. Murray, S. D. Putney, and G. Gray.** 1992. Mutations in the principal neutralization determinant of human immunodeficiency virus type 1 affect syncytium formation, virus infectivity, growth kinetics, and neutralization. *J. Virol.* **66**:1875–1883.
 27. **Hallenberger, S., V. Bosch, H. Angliker, E. Shaw, H.-D. Klenk, and W. Garten.** 1992. Inhibition of furin-mediated cleavage activation of HIV-1 glycoprotein gp160. *Nature (London)* **360**:358–361.
 28. **Hart, T., R. Kirsch, H. Ellens, R. Sweet, D. Lambert, S. Petteway, J. Leary, and P. Bugelski.** 1991. Binding of soluble CD4 proteins to HIV-1 and infected cells induces release of envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **88**:2189–2193.
 29. **Helseth, E., M. Kowalski, D. Gabuzda, U. Olshevsky, W. Haseltine, and J. Sodroski.** 1990. Rapid complementation assays measuring replicative potential of human immunodeficiency virus type 1 envelope glycoprotein mutants. *J. Virol.* **64**:2416–2420.
 30. **Helseth, E., U. Olshevsky, C. Furman, and J. Sodroski.** 1991. Human immunodeficiency virus type 1 gp120 envelope glycoprotein regions important for association with the gp41 transmembrane glycoprotein. *J. Virol.* **65**:2119–2123.
 31. **Helseth, E., U. Olshevsky, D. Gabuzda, B. Ardman, W. Haseltine, and J. Sodroski.** 1990. Changes in the transmembrane region of the human immunodeficiency virus type 1 gp41 envelope glycoprotein affect membrane fusion. *J. Virol.* **64**:6314–6318.
 32. **Horvath, C. M., R. G. Paterson, M. A. Shaughnessy, R. Wood, and R. A. Lamb.** 1992. Biological activity of paramyxovirus fusion proteins: factors influencing formation of syncytia. *J. Virol.* **66**:4564–4569.
 33. **Hu, X., R. Ray, and R. W. Compans.** 1992. Functional interactions between the fusion protein and hemagglutinin-neuraminidase of human parainfluenza viruses. *J. Virol.* **66**:1528–1534.
 34. **Ivanoff, L., D. Looney, C. McDanal, J. Morris, F. Wong-Staal, A. Langlois, S. Petteway, and T. Matthews.** 1991. Alteration of HIV-1 infectivity and neutralization by a single amino acid replacement in the V3 loop domain. *AIDS Res. Hum. Retroviruses* **7**:595–603.
 - 34a. **Ivey-Hoyle, M., R. K. Clark, and M. Rosenberg.** 1991. The N-terminal 31 amino acids of human immunodeficiency virus type 1 envelope protein gp120 contain a potential gp41 contact site. *J. Virol.* **65**:2682–2685.
 35. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature (London)* **312**:767–768.
 36. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. G. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the human immunodeficiency virus envelope glycoproteins. *Science* **237**:1351–1355.
 37. **Lasky, L., G. Nakamura, D. Smith, C. Shimisaki, E. Patzer, and P. Berman.** 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.
 38. **Lifson, J., M. Feinberg, G. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. Steimer, and E. Engleman.** 1986. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Science* **233**:725–728.
 39. **Maddon, P., A. Dalgleish, J. S. McDougal, P. Clapham, R. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**:333–348.
 40. **McCune, J., L. Rabin, M. Feinberg, M. Lieberman, J. Kosek, G. Reyes, and I. Weissman.** 1988. Endoproteolytic cleavage of gp160 is required for the activation of human immunodeficiency virus. *Cell* **53**:55–67.
 41. **McDougal, J. S., M. Kennedy, J. Sligh, S. Cort, A. Mowie, and J. Nicholson.** 1986. Binding of the HTLV-III/LAV to T4⁺ T cells by a complex of the 100 K viral protein and the T4 molecule. *Science* **231**:382–385.
 42. **McDougal, J. S., J. Nicholson, G. Cross, S. Cort, M. Kennedy, and A. Mawle.** 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J. Immunol.* **137**:2937–2944.
 43. **Moore, J., J. McKeating, R. Weiss, and Q. Sattentau.** 1990. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. *Science* **250**:1139–1142.
 44. **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.
45. Morrison, T., C. McQuain, and L. McGinnes. 1991. Complementation between avirulent Newcastle disease virus and a fusion protein gene expressed from a retrovirus vector: requirements for membrane fusion. *J. Virol.* **65**:813–822.
46. 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.
47. Page, K. A., S. M. Stearns, and D. R. Littman. 1992. Analysis of mutations in the V3 domain of gp160 that affect fusion and infectivity. *J. Virol.* **66**:524–533.
48. Paterson, R. G., S. W. Hiebert, and R. A. Lamb. 1985. Expression at the cell surface of biologically active fusion and hemagglutinin/neuraminidase proteins of the paramyxovirus simian virus 5 from cloned cDNA. *Proc. Natl. Acad. Sci. USA* **82**:7520–7524.
49. Perez, L. G., M. A. O'Donnell, and E. B. Stephens. 1992. The transmembrane glycoprotein of human immunodeficiency virus type 1 induces syncytium formation in the absence of the receptor binding glycoprotein. *J. Virol.* **66**:4134–4143.
50. Pinter, A., W. J. Honnen, S. A. Tilley, C. Bona, H. Zaghoulani, 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.
- 50a. Poznansky, M. Unpublished data.
51. 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.
52. Robey, W. G., B. Safai, S. Oroszlan, L. Arthur, M. Gonda, R. Gallo, and P. J. Fischinger. 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. *Science* **228**:593–595.
53. Sattentau, Q., and J. Moore. 1991. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. *J. Exp. Med.* **174**:407–415.
- 53a. Sattentau, Q. J., J. P. Moore, F. Vignaux, F. Traincard, and P. Poignard. 1993. Conformational changes induced in the envelope glycoproteins of the human and simian immunodeficiency viruses by soluble receptor binding. *J. Virol.* **67**:7383–7393.
54. Schawaller, M., C. Smith, J. Skehel, and D. Wiley. 1989. Studies with cross-linking reagents on the oligomeric structure of the *env*-glycoprotein of HIV. *Virology* **172**:367–369.
55. Sodroski, J., W. C. Goh, C. A. Rosen, K. Campbell, and W. Haseltine. 1986. Role of the HTLV-III envelope in syncytium formation and cytopathicity. *Nature (London)* **321**:412–417.
56. Spear, G. T., D. M. Takefman, B. L. Sullivan, A. L. Landay, and S. Zolla-Pazner. 1993. Complement activation by human monoclonal antibodies to human immunodeficiency virus. *J. Virol.* **67**:53–59.
57. Stein, B., S. Gouda, J. Lifson, R. Penhallow, K. Bensch, and E. Engleman. 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. *Cell* **49**:659–668.
58. Stein, B. S., and E. G. Engleman. 1990. Intracellular processing of the gp160 HIV-1 envelope precursor: endoproteolytic cleavage occurs in a *cis* or medial compartment of the Golgi complex. *J. Biol. Chem.* **265**:2640–2649.
59. Sullivan, N., M. Thali, C. Furman, D. D. Ho, and J. Sodroski. 1993. Effect of amino acid changes in the V1/V2 region of the human immunodeficiency virus type 1 gp120 glycoprotein on subunit association, syncytium formation, and recognition by a neutralizing antibody. *J. Virol.* **67**:3674–3679.
60. Thali, M., C. Furman, E. Helseth, H. Repke, and J. Sodroski. 1992. Lack of correlation between soluble CD4-induced shedding of the human immunodeficiency virus type 1 exterior envelope glycoprotein and subsequent membrane fusion events. *J. Virol.* **66**:5516–5524.
61. Travis, B., T. Dykers, D. Hewgill, J. Ledbetter, T. Tsu, S.-L. Hiu, and J. Lewis. 1992. Functional roles of the V3 hypervariable region of HIV-1 gp160 in the processing of gp160 and in the formation of syncytia in CD4+ cells. *Virology* **186**:313–317.
62. Veronese, F. D., A. Vico, T. Copeland, S. Oroszlan, R. Gallo, and M. Sarngadharan. 1985. Characterization of gp41 as the transmembrane protein encoded by the HTLV-III/LAV envelope gene. *Science* **229**:1402–1405.
63. Wild, C., T. Oas, C. McDanal, D. Bolognesi, and T. Matthews. 1992. A synthetic peptide inhibitor of human immunodeficiency virus replication: correlation between solution structure and viral inhibition. *Proc. Natl. Acad. Sci. USA* **89**:10537–10541.
64. Wild, T. F., E. Malvoisin, and R. Buckland. 1991. Measles virus: both the haemagglutinin and fusion glycoproteins are required for fusion. *J. Gen. Virol.* **72**:439–442.
65. Willey, R. L., J. S. Bonifacino, B. J. Potts, M. A. Martin, and R. D. Klausner. 1988. Biosynthesis, cleavage, and degradation of the human immunodeficiency virus 1 envelope glycoprotein gp160. *Proc. Natl. Acad. Sci. USA* **85**:9580–9584.
66. 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.
67. Xu, J.-Y., M. K. Gorny, T. Palker, S. Karwowska, and S. Zolla-Pazner. 1991. Epitope mapping of two immunodominant domains of gp41, the transmembrane protein of human immunodeficiency virus type 1, using ten human monoclonal antibodies. *J. Virol.* **65**:4832–4838.