JOURNAL OF VIROLOGY, Sept. 1992, p. 5516–5524
0022-538X/92/095516-09$02.00/0

Vol. 66, No. 9

Lack of Correlation between Soluble CD4-Induced Shedding of the Human Immunodeficiency Virus Type 1 Exterior Envelope Glycoprotein and Subsequent Membrane Fusion Events

MARKUS THALI, CRAIG FURMAN, EIRIK HELSETH, HEINRICH REPKE, AND JOSEPH SODROSKI*

Division of Human Retrovirology, Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Received 6 April 1992/Accepted 17 June 1992

The noncovalent association of the gp120 and gp41 envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1) is disrupted by soluble CD4 binding, resulting in shedding of the gp120 exterior envelope glycoprotein. This observation has led to the speculation that interaction of gp120 with the CD4 receptor triggers shedding of the exterior envelope glycoprotein, allowing exposure of gp41 domains necessary for membrane fusion steps involved in virus entry or syncytium formation. To test this hypothesis, a set of HIV-1 envelope glycoprotein mutants were used to examine the relationship of soluble CD4-induced shedding of the gp120 glycoprotein to envelope glycoprotein function in syncytium formation and virus entry. All mutants with a threefold or greater reduction in CD4-binding ability exhibited marked decreases in gp120 shedding in response to soluble CD4, even though several of these mutants exhibited significant levels of envelope glycoprotein function. Conversely, most fusion-defective mutants with wild-type gp120-CD4 binding affinity, including those with changes in the V3 loop, efficiently shed gp120 following soluble CD4 binding. Thus, soluble CD4-induced shedding of gp120 is not a generally useful marker for conformational changes in the HIV-1 envelope glycoproteins necessary for the virus entry or syncytium formation processes. Some gp120 mutants, despite being expressed on the cell surface and capable of efficiently binding soluble CD4, exhibited decreased gp120 shedding. These mutants were still sensitive to neutralization by soluble CD4, indicating that, for envelope glycoproteins exhibiting high affinity for soluble CD4, competitive inhibition may be more important than gp120 shedding for the antiviral effect.

Human immunodeficiency virus type 1 (HIV-1) is the cause of AIDS, which is characterized by the depletion of CD4-positive lymphocytes (6, 22, 26). HIV-1 exhibits a tropism for CD4-bearing cells that is mediated by a specific interaction between the viral envelope glycoproteins and the CD4 glycoprotein (20, 37, 43, 45). The HIV-1 envelope glycoproteins are synthesized as a 160-kDa precursor (gp160) that is cleaved into the exterior envelope glycoprotein (gp120), which binds to the CD4 molecule, and a transmembrane glycoprotein (gp41) (1, 57). The HIV-1 gp120 and gp41 glycoproteins remain associated through noncovalent interactions (30, 39, 45a). Following CD4 binding, the envelope glycoproteins mediate a pH-independent process that results in the fusion of the viral and target cell membranes and that is necessary for virus entry (29, 65). HIV-1 envelope glycoproteins expressed on the surface of infected cells initiate receptor-binding and membrane fusion events with adjacent CD4-positive cells, resulting in the formation of multinucleated syncytia (42, 63).

Structural and mutagenic studies have provided some insight into the molecular events underlying HIV-1 entry and syncytium formation. The CD4-binding region of gp120 consists of a conformation-dependent structure with elements derived from discontinuous sequences in the third and fourth conserved regions of the glycoprotein (17, 18, 39, 41, 52). The gp120 glycoprotein binds to the CD4 region bearing similarity to the second complementarity-determining (CDR2) region of immunoglobulins (3, 4, 10, 12, 16, 35, 40, 47, 54). The CDR2-like region of CD4, located in the amino-terminal domain of the glycoprotein, consists of a hydrophobic ridge protruding from a positively charged surface (59, 70). Mutagenic studies have indicated that the gp120-binding site is discontinuous but spatially limited to the CD4 region near phenylalanine 43, lysine 46, and arginine 59 (15).

Amino acid changes in specific regions of both the gp120 and gp41 proteins result in envelope glycoproteins that retain receptor-binding ability but that are fusion defective. These regions include (i) the third variable (V3) loop of gp120, which is a target for neutralizing antibodies (25, 27, 29, 33, 44, 56, 58, 61, 69); (ii) the gp41 amino terminus (24, 29, 38, 39); and (iii) the gp41 transmembrane region (31). The analogous position of hydrophobic, fusogenic domains on the amino termini of transmembrane glycoproteins of several enveloped viruses suggest functional similarities in the membrane fusion process (for reviews, see references 64, 71, and 72). It has been proposed that for viruses like HIV-1 that fuse membranes at neutral pH, binding to the viral receptor predisposes to conformational changes in the envelope glycoproteins analogous to those induced by acid pH, which triggers the entry of orthomyxoviruses (64, 71, 72). In the latter case, these changes result in exposure of the hydrophobic "fusion peptide" at the amino terminus of the hemagglutinin (HA_2) transmembrane glycoprotein, perhaps favoring HA_2 interaction with the membrane(s) involved in the fusion process (72). Recent studies have provided ge-

* Corresponding author.

netic evidence that the amino terminus of the HIV-1 gp41 glycoprotein may interact with the target cell during the membrane fusion event (9). A number of studies have suggested that interaction with the CD4 glycoprotein can cause conformational alterations in the HIV-1 envelope glycoproteins, but whether any of the described alterations represents a necessary step in the virus entry process has not been addressed (28, 50, 60). Following the 4°C binding of gp120 and CD4, a higher affinity interaction can be observed when the temperature is shifted to 37°C (49, 66). A soluble form of the CD4 molecule, developed as a potential anti-HIV-1 therapy (7, 21, 23, 32, 62, 69), induces the dissociation of the gp120 and gp41 glycoproteins at 37°C but not at 4°C (28, 50). Synthetic peptides corresponding to the CDR3-like region of CD4 and polyanions like dextran sulfate also induce the shedding of the gp120 glycoprotein from the envelope glycoprotein complex (8, 13). Since the latter agents have been shown to interact with the V3 loop of gp120 (14), it has been proposed that the V3 loop and/or the CDR3-like region of CD4 may play a role in gp120 dissociation (5, 8). This would then allow exposure of hydrophobic gp41 determinants necessary for the membrane fusion process. Here we test this hypothesis by examining the relationship between soluble CD4-induced gp120 dissociation and the fusogenic capacity of a panel of HIV-1 envelope glycoprotein mutants.

MATERIALS AND METHODS

Envelope glycoprotein expression and CD4 binding assay. COS-1 cells were transfected by the DEAE-dextran method with pSVIIIenv DNA expressing wild-type or mutant envelope glycoproteins as described previously (29, 52). In some experiments, a *tat*-expressing plasmid was cotransfected to increase the level of envelope glycoprotein expression. To measure expression, the cells were radiolabeled with [^{35}S] cysteine overnight and precipitated with an excess of a mixture of sera derived from AIDS patients (29, 52). Expression of the mutant glycoproteins, processing of the glycoprotein precursor, association of gp41 and gp120 glycoproteins, and ability of the supernatant gp120 to bind CD4-positive SupT1 lymphocytes were determined as described previously (29, 30, 52). The pSVIIIenvΔKS plasmid, which contains a frameshift and deletion within the *env* gene, was used as a negative control.

Cell surface expression of envelope glycoproteins. The expression of the mutant glycoproteins on the surface of transfected COS-1 cells was measured by using three assays.

(i) Radiolabeled anti-gp120 antibody. COS-1 cells were transfected with 10 μg of pSVIIIenv DNA expressing wild-type or mutant envelope glycoproteins. Sixty hours after transfection, the COS-1 cells were washed with phosphate-buffered saline (PBS) containing 2% fetal bovine serum and incubated with a ^{35}S-labeled monoclonal antibody against gp120, F105. F105 antibody-producing hybridomas were obtained from Marshall Posner (55), and 10^8 cells were labeled overnight in 80 ml of cysteine- and methionine-free RPMI medium containing 5 mCi each of [^{35}S]cysteine and [^{35}S]methionine. One milliliter of the F105 antibody-containing medium was added to each 100-mm dish of transfected COS-1 cells. The envelope-expressing COS-1 cells were incubated with the antibody for 1 h at 4°C, washed three times with PBS containing 2% fetal bovine serum, and lysed. The lysates were incubated with protein A-Sepharose beads, which were washed according to the procedure used for immunoprecipitation (39). The labeled antibody was visualized on a reducing sodium dodecyl sulfate (SDS)-polyacrylamide gel.

(ii) Binding of serum antibodies from an AIDS patient to envelope glycoprotein-expressing COS-1 cells. COS-1 cells transfected with an envelope expressor plasmid were metabolically labeled with [^{35}S]cysteine for 16 h at 48 h posttransfection. The intact labeled cells were washed twice with PBS containing 2% heat-inactivated fetal bovine serum, incubated with a 1:100 dilution of an AIDS patient's serum reactive with the envelope glycoproteins in 0.5 ml of PBS–2% fetal bovine serum at 4°C for 60 min, rinsed three times with PBS containing 2% fetal bovine serum, and lysed in RIPA lysis buffer at 4°C. The cell lysates were clarified by ultracentrifugation, and bound envelope glycoproteins were immunoprecipitated by incubation with protein A-Sepharose as described above.

(iii) Iodination of cell surface envelope glycoproteins by lactoperoxidase. For some of the mutant envelope glycoproteins, the envelope glycoproteins on the cell surface were examined by iodination followed by immunoprecipitation with an AIDS patient's serum. A 100-mm dish of transfected COS-1 cells was washed four times with ice-cold PBS. Then, 900 μl of PBS, followed by 100 μl of lactoperoxidase (100 U/ml in PBS; Calbiochem), 0.5 mCi of ^{125}I (Amersham), and 25 μl of a 1:1,000 (vol/vol) solution of 30% hydrogen peroxide in distilled water, was added to the dish. An additional 25 μl of the hydrogen peroxide solution was added after 5, 10, and 15 min. After 20-min total incubation time at room temperature, the cells were lysed and used for immunoprecipitation with a mixture of sera derived from AIDS patients.

Functional assays for the HIV-1 envelope glycoproteins. The ability of the mutant glycoproteins to mediate the formation of syncytia was assessed by cocultivating transfected COS-1 cells with SupT1 CD4-positive lymphocytes as previously described (9, 66). All reported values for syncytium-forming ability represent the mean of at least two independent experiments, with the differences between experiments not exceeding 20% of the value reported. Complementation of a single round of replication of the *env*-deficient chloramphenicol acetyltransferase (CAT)-expressing provirus by the mutant envelope glycoproteins was performed as described previously (29). For mutants for which soluble CD4 inhibition of syncytium formation was measured, soluble CD4 was incubated with the envelope glycoprotein-expressing cells for 1 h at 37°C prior to addition of SupT1 cells, as described previously (66). For soluble CD4 inhibition of virus replication, soluble CD4 was incubated with recombinant CAT-expressing virus for 1 h at 37°C before exposure of the virus to target Jurkat lymphocytes. CAT activity in the target cells was measured as described previously (66).

Soluble CD4-induced shedding of gp120. COS-1 cells transfected with the envelope glycoprotein-expressing plasmids were labeled with [^{35}S]cysteine (200 μCi in 4 ml of cysteine-free medium) overnight at 48 h following transfection. The labeled cells were then washed and incubated with either 0, 1, 10, or 30 μg of soluble CD4 (0, 20, 200, or 600 nM) (American BioTechnologies, Inc.) per ml in PBS for 1 h at 37°C. The cell supernatants and cell lysates (in RIPA buffer) were then precipitated with a mixture of sera from AIDS patients.

Soluble CD4 binding to cell surface envelope glycoprotein complexes. Transfected COS-1 cells were incubated with 10^{-8} or 3.3×10^{-9} M soluble CD4 that had been iodinated by the Bolton-Hunter method to a specific activity of 53 Ci/mmol. These concentrations were calculated on the basis of the estimated fraction of native soluble CD4 in the iodinated

TABLE 1. Summary of results for wild-type and mutant glycoproteins

Glycoprotein	Processing index[a]	Association index[a]	CD4-binding ability[a]	Cell surface expression[b]		Soluble CD4-induced dissociation[c]	Syncytium formation	Replication complementation (mock = 4)
				F105 binding	Patient's serum binding			
Wild type	1.0	1.0	1.0	+++	+++	+++	100	100
125 L/G	1.0	0.55	1.3	+++	ND[d]	+++	10	27
257 T/R	0.43	1.0	0.16	−[e]	+++	−	5	28
257 T/A	0.54	1.2	1.1	ND	+++	+++	64	98
257 T/G	0.38	1.2	1.0	ND	+++	++	59	45
308/9/10	0.65	0.63	WT[f]	++	+++	+++	<1	2
313 P/S	0.99	0.90	1.0	+++	ND	+++	72	34
314 G/Q	1.0	1.0	0.93	+++	+++	+++	<1	4
368 D/T	0.86	0.93	0.33	−[e]	+++	−	16	50
368 D/E	1.0	0.81	0.09	ND	+++	−	51	70
368 D/P	0.94	0.91	0.09	−[e]	+++	−	<1	23
370 E/Q	0.64	1.0	0.018	ND	+++	−	<1	33
382 F/L	1.0	0.20	2.7	+++	+++	++	2	34
420 I/R	0.82	0.16	1.2	+++	+++	+	3	32
430 V/S	0.93	1.0	0.54	ND	+++	++	80	100
433 A/L	0.83	0.43	1.7	++	+++	+	2	43
438 P/R	1.0	0.26	2.3	++	+++	+	2	19
456 R/K	0.55	1.0	0.41	ND	+++	+++	75	ND
457 D/R	0.60	0.84	0.15	+[e]	ND	−	<1	8
457 D/A	0.88	0.76	0.09	ND	+++	−	47	36
457 D/E	WT	WT	0.38	ND	+++	+	66	89
477 D/V	0.25	1.0	0.39	+[e]	ND	++	50	42
589 D/L	2.2	0.26	WT	++	ND	+++	<1	6
652 Q/L	WT	WT	WT	+++	ND	+++	100	142

[a] These values were previously reported in references 30 and 52. The CD4-binding ability represents the binding of radiolabeled mutant gp120 glycoprotein to SupT1 CD4-positive lymphocytes, relative to the value observed for the wild-type gp120 glycoprotein (52).

[b] Cell surface expression was evaluated by using F105 binding or an AIDS patient's serum. Binding: +++, wild-type levels; ++, 50 to 90% of wild-type levels; +, 10 to 49% of wild-type levels; −, <10% of wild-type levels.

[c] +++, wild-type levels of gp120 shed in response to all concentrations of soluble CD4; ++, shedding occurs at slightly less than wild-type levels; +, shedding occurs but at significantly reduced levels compared with that of the wild type; −, no observed shedding of gp120 at 30 μg of soluble CD4 per ml.

[d] ND, not determined.

[e] Levels expected to be decreased because of decreased recognition of this mutant glycoprotein by F105 antibody.

[f] WT, these values were estimated to be near wild-type levels.

fraction and were chosen to be near the dissociation constant for the soluble CD4-cell surface HIV-1 envelope glycoprotein interaction. Use of these soluble CD4 concentrations allows maximum sensitivity with respect to changes in affinity. Incubations were carried out at 37°C for 90 min, after which the cells were washed three times in PBS, lysed, and immunoprecipitated with OKT4 antibody. Bound soluble CD4 was visualized on an SDS-polyacrylamide gel.

RESULTS

Processing and cell surface expression of the mutant glycoproteins. The panel of mutants of the HIV-1 (HXBc2 strain) envelope glycoproteins has been previously described (30, 52, 67). The processing of the gp160 precursor glycoprotein, the association of the gp120 and gp41 subunits, the CD4-binding ability, and the functional activity in syncytium formation or virus replication assays have been reported for some of these mutants (29, 30, 52, 66, 67). Most of the mutants selected for this study exhibited only modest changes in gp160 precursor processing relative to that observed for the wild-type glycoprotein (Table 1). The steady-state levels of envelope glycoprotein expression on the surface of transfected COS-1 cells were examined by three methods. For mutants that were recognized equivalently to the wild-type glycoprotein by the F105 monoclonal antibody (55), which recognizes a discontinuous gp120 epitope near the CD4-binding region (68), the binding of radiolabeled F105 antibody to the transfected COS-1 cells expressing no envelope glycoproteins, the wild-type HXBc2 envelope glycoproteins, or the mutant glycoproteins was measured. The binding of the F105 antibody to COS-1 cells expressing some of the mutant envelope glycoproteins is shown in Fig. 1. No F105 binding was observed for COS-1 cells transfected with the pSVIIIenvΔKS plasmid, which contains a deletion and frameshift mutation within the *env* gene. The level of F105 binding was equivalent to that of the wild-type glycoproteins for most of the mutants previously shown to be recognized by the F105 antibody. The level of F105 binding was reduced or absent for the envelope glycoproteins containing changes in threonine 257, aspartic acid 368, aspartic acid 457, or aspartic acid 477, as expected from previous studies showing that these amino acid changes affected F105 recognition of the gp120 glycoprotein (67).

For mutant glycoproteins that were not recognized by the F105 antibody equivalently to the wild-type glycoprotein, the steady-state level of cell surface expression in the transfected COS-1 cells was measured by incubating the radiolabeled transfected cells with a polyclonal mixture of HIV-positive sera. The cells were then washed, and the amount of bound antibody was determined by lysing the cells, adding protein A-Sepharose beads, and immunoprecipitating the labeled envelope glycoproteins. The results of both assays for cell surface expression of the mutant envelope glycoproteins are summarized in Table 1.

The above assays for cell surface expression provide data on the amounts of the envelope glycoproteins on the cell

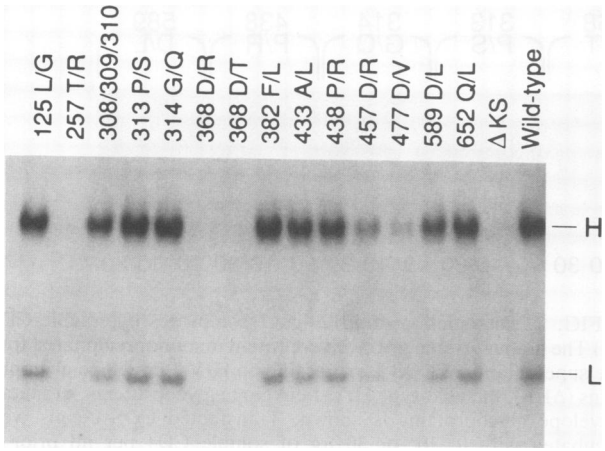

FIG. 1. Cell surface expression of envelope glycoprotein mutants. Shown are results for binding of radiolabeled F105 antibody to COS-1 cells expressing no HIV-1 envelope glycoproteins (ΔKS), the wild-type HIV-1 glycoproteins, or mutant envelope glycoproteins, as indicated above each lane. The positions of the heavy (H) and light (L) antibody chains are indicated.

surface but do not distinguish gp160 from gp120 glycoproteins. Since some of the mutants with amino acid changes in the C3 or C4 region exhibit a partial dissociation of the glycoprotein subunits (30), iodination of transfected cells was carried out to assess the proportion of gp160 and gp120 glycoproteins on the COS-1 cell surface. These studies indicated that, for the wild-type HIV-1 envelope glycoproteins, mostly the gp120 glycoprotein but also lower levels of the gp160 precursor glycoprotein were expressed on the transfected COS-1 surface. When higher levels of envelope glycoprotein expression were achieved, as for example by cotransfection of a *tat*-expressing plasmid, the proportion of the gp160 glycoprotein on the cell surface increased. The levels and proportions of the gp160 and gp120 glycoproteins were comparable for the 420 I/R, 433 A/L, and 438 P/R mutants and the wild-type envelope glycoproteins (data not shown). Apparently, only a minor fraction of the cell-associated gp120 glycoprotein is present on the cell surface; thus, even though these mutants exhibit partial dissociation of gp120 and gp41 glycoproteins, sufficient intact envelope glycoproteins remain and are channeled to the cell surface to achieve levels there comparable to those of the wild-type glycoproteins. In summary, despite differences in precursor processing or subunit association, most of the mutants used in this study are expressed on the cell surface comparably to the wild-type proteins.

Function of the glycoprotein mutants. To assess the function of the mutant envelope glycoproteins, syncytium-forming and replicative abilities were determined. Table 1 shows that, as previously reported (66), several HIV-1 mutants with significant decreases in CD4-binding ability were still competent in forming syncytia or mediating virus entry. A second group of mutants, with amino acid changes in the third variable loop of the gp120 glycoprotein, were attenuated for these functions despite exhibiting approximately wild-type levels of precursor processing, subunit association, and CD4 binding. A third group of mutants exhibited decreases in subunit association and, despite adequate levels of cell surface expression and receptor binding ability, were fusion defective and at least partly deficient in virus entry. These amino acid changes involve the base of the V1/V2 loop structure (125 L/G), the hydrophobic components of the third and fourth conserved gp120 regions (382 F/L, 420 I/R, 433 A/L, 438 P/R), and the gp41 ectodomain (589 D/L). Changes in the last two regions have previously been shown to alter the affinity of the noncovalent gp120-gp41 interaction (30, 39).

Soluble CD4-induced shedding of the gp120 glycoprotein. To determine whether a relationship exists between soluble CD4-induced shedding of the gp120 glycoprotein and the function of the envelope glycoproteins, the induction of gp120-gp41 dissociation by soluble CD4 was measured for the wild-type and mutant envelope glycoproteins by incubating metabolically labeled transfected COS-1 cells with different concentrations of soluble CD4 for 1 h at 37°C. The amount of labeled gp120 glycoprotein shed into the supernatant was then determined by precipitation with a polyclonal mixture of HIV-positive sera. The results are shown in Fig. 2 and summarized in Table 1. A significant increase (approximately 40-fold) in the amount of the wild-type gp120 glycoprotein was observed following soluble CD4 treatment. All of the mutant glycoproteins (257 T/R, 368 D/T, 368 D/E, 368 D/P, 370 E/Q, 457 D/R, and 457 D/A) with relative CD4-binding abilities of 0.38 or less exhibited significant reductions in the amount of gp120 shed in response to soluble CD4 treatment. Several of these mutants exhibited significant competence at forming syncytia or mediating virus entry, indicating that soluble CD4-induced shedding is unlikely to be a necessary component of envelope glycoprotein function.

Most of the mutant glycoproteins with relative CD4-binding abilities greater than 0.38 exhibited gp120 shedding following soluble CD4 incubation at levels comparable to that seen for the wild-type glycoproteins. This was the case for several fusion-defective mutants, including those with alterations in the V3 loop or the gp41 ectodomain (125 L/G, 308/9/10 RIQ/RPELIPVQ, 313 P/S, 314 G/Q, and 589 D/L).

One mutant glycoprotein, 257 T/G, exhibited some decrease in soluble CD4-induced gp120 shedding despite retaining approximately wild-type CD4-binding ability (Fig. 2B). The latter was determined by comparing the ability of monomeric, soluble gp120 glycoproteins to bind CD4-positive SupT1 lymphocytes (52). The relative CD4-binding ability of the 257 T/G mutant was comparable to that of the 257 T/A mutant, which exhibited wild-type levels of gp120 shedding in response to soluble CD4 treatment. To examine whether the 257 T/G mutant in the multimeric envelope glycoprotein complex on the cell surface binds soluble CD4 comparably to that of the wild-type or 257 T/A glycoproteins, the binding of radiolabeled soluble CD4 to transfected COS-1 cells was assessed. These studies (Fig. 3) indicate a lower binding affinity of the 257 T/G mutant, as it exists on the cell surface, relative to that of either the wild-type or 257 T/A glycoproteins. To examine whether this change in affinity resulted in a change of soluble CD4 sensitivity, the ability of soluble CD4 to inhibit syncytium formation or virus entry of the 257 T/G mutants was compared to that of the wild-type or 257 T/A glycoproteins (Fig. 4A). As previously reported (66), for both syncytium induction and virus entry, the 257 T/A mutant was as sensitive to soluble CD4 as were the wild-type glycoproteins. By contrast, both the syncytium inhibition and virus neutralization curves (Fig. 4B) for the 257 T/G mutant exhibited a "shoulder," with decreased sensitivity to the intermediate range of soluble CD4 concentrations. Thus, a mild change in affinity of the multimeric glycoprotein complex, even in the apparent absence of changes in monomeric gp120-CD4 binding affinity, can alter

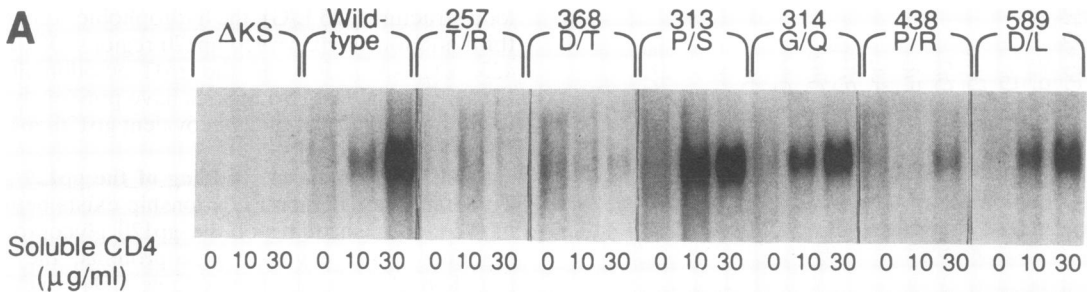

FIG. 2. Dissociation of gp120-gp41 complexes by soluble CD4. (A) The amount of the gp120 glycoprotein immunoprecipitated from the supernatants of COS-1 expressing no HIV-1 envelope glycoproteins (ΔKS), the wild-type HIV-1 envelope glycoproteins, or mutant envelope glycoproteins is shown. Transfected COS-1 cells were incubated with 0, 10, or 30 μg of soluble CD4 per ml prior to immunoprecipitation of the supernatants. (B) The amount of gp120 glycoprotein precipitated from supernatants of COS-1 cells expressing either the wild-type glycoproteins or mutant glycoproteins with changes in threonine 257 following soluble CD4 incubation is shown.

the sensitivity of the functional glycoproteins to soluble CD4.

A group of mutants with amino acid changes in the C3 and C4 hydrophobic regions (382 F/L, 420 I/R, 433 A/L, and 438 P/R) exhibited decreased shedding of gp120 following soluble CD4 treatment, relative to that observed for the wild-type envelope glycoproteins. These mutant monomeric gp120 glycoproteins all bound CD4 as efficiently as the wild-type glycoprotein (Table 1), and radiolabeled soluble CD4 binding to the mutant glycoprotein complexes on the COS-1 cell surface exhibited affinities comparable to that of the wild-type glycoproteins (Fig. 3). Thus, these mutants apparently bind soluble CD4 with high affinity but are specifically resistant to gp120 shedding. Syncytium-forming ability of these mutants was too low to determine whether the mutants exhibited changes in soluble CD4 inhibition of cell-cell fusion. Although the replicative ability of these fusion-deficient mutants was low, sufficient activity was retained to allow assessment of soluble CD4 neutralization in the complementation assay. As shown in Fig. 4B, all of these mutants were sensitive to soluble CD4 inhibition of virus entry.

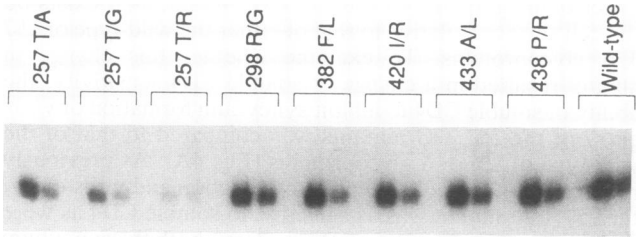

FIG. 3. Binding of soluble CD4 to the cell surface envelope glycoprotein complex. The amount of labeled soluble CD4 bound to the surface of COS-1 cells expressing either the wild-type glycoproteins or mutant glycoproteins is shown. Cells were incubated with either 10^{-8} M (left lane) or 3.3×10^{-9} M (right lane) labeled soluble CD4. No binding was observed for COS-1 cells that were mock transfected.

DISCUSSION

This study examined the proposed relationship among soluble CD4-induced gp120-gp41 dissociation, the antiviral effects of soluble CD4, and conformational changes in the HIV-1 envelope glycoproteins postulated to be essential for the membrane fusion process. The inclusion of a significant number of HIV-1 envelope glycoprotein mutants allows an assessment of whether a correlation exists among these phenomena. The results of this study clearly indicate a strong relationship between CD4-binding affinity and soluble CD4-induced shedding of the gp120 glycoprotein. All gp120 mutants with a relative CD4-binding ability less than or equal to 0.38 exhibited dramatic decreases in gp120 shedding following soluble CD4 incubation. Thus, the attainment of a high-affinity interaction between soluble CD4 and the HIV-1 envelope glycoproteins would appear to represent one requirement for gp120-gp41 dissociation. The binding of the HIV-1 gp120 glycoprotein and CD4 has been shown to consist of two processes, an initial interaction that occurs at 4°C and a temperature-dependent "induced fit," which results in a six- to ninefold increase in affinity (49, 66). The contribution of the induced fit to the final affinity of the gp120-CD4 interaction probably accounts for the temperature dependence observed for the induction of gp120 shedding by soluble CD4 (49, 50). This assertion is supported by the observation that gp120 mutants previously shown not to undergo the temperature-dependent increase in CD4-binding affinity (257 T/R, 368 D/T, 368 D/P, 368 D/E, 370 E/Q, 457 D/R, and 457 D/A) (66) are also less efficient in shedding gp120 following soluble CD4 exposure.

Sensitivity to soluble CD4-mediated gp120 shedding, as is the case for the induced fit reaction (66), does not correlate with the ability of mutant envelope glycoproteins to mediate the membrane fusion process. This assertion follows from the observation that a number of gp120 mutants with decreased sensitivity to soluble CD4-mediated gp120-gp41 dissociation retain a high level of function with respect to either syncytium formation or virus entry. This idea is consistent

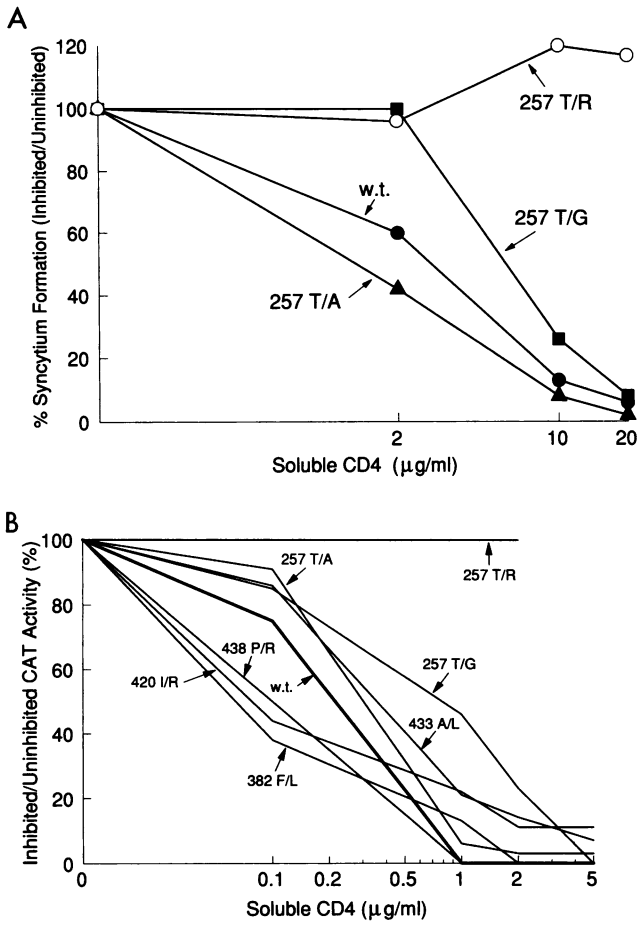

FIG. 4. Inhibition of mutant envelope glycoprotein function by soluble CD4. (A) Soluble CD4 inhibition of syncytia induced by the wild-type (w.t.) and mutant envelope glycoproteins expressed on COS-1 cells, which were cocultivated with SupT1 lymphocytes, is shown. The percentage of syncytia observed in the presence of the indicated concentration of soluble CD4 relative to the number observed in the absence of soluble CD4 treatment is shown. (B) Inhibition of virus entry of recombinant viruses expressing the CAT gene by soluble CD4, for viruses with either wild-type (w.t.) or mutant HIV-1 envelope glycoproteins, is shown. The percentage of CAT activity observed in the presence of a given concentration of soluble CD4 relative to the CAT activity observed in the absence of soluble CD4 is shown. The experiments shown are representative and were repeated twice with comparable results.

with the previously reported tolerance of naturally occurring and laboratory-produced HIV-1 variants for lowered gp120-CD4 affinity (34, 66). It is also consistent with the observation that many replication-competent isolates from HIV-1 patients, as well as other primate and human immunodeficiency viruses, are resistant to the antiviral effects, and presumably the induction of envelope glycoprotein subunit dissociation, associated with soluble CD4 (1, 19, 51).

For mutant HIV-1 envelope glycoproteins with wild-type CD4-binding ability, no clear relationship between soluble CD4-induced gp120 shedding and membrane fusion potential was observed. Several mutant glycoproteins (125 L/G, 308/9/10 RIQ/RPELIPVQ, 314 G/Q, and 589 D/L) partially or significantly impaired in elements of the membrane fusion process shed gp120 following soluble CD4 treatment as efficiently as did the wild-type glycoproteins. In other studies, fusion-defective HIV-1 mutants with changes in the gp41 amino terminus were also shown to be as sensitive to the antiviral effects of soluble CD4 as were the wild-type glycoproteins (9). Thus, the reliability of soluble CD4-induced gp120-gp41 dissociation as a marker for conformational changes in the HIV-1 envelope glycoproteins necessary for the fusion of membranes could not be documented in this study.

The observation that membrane fusion-defective gp120 mutants with amino acid alterations in the V3 loop efficiently shed gp120 following soluble CD4 treatment clearly indicates that the role of the V3 loop in syncytium formation or virus entry is separable from any proposed role in the dissociation of gp120 and gp41 induced by receptor binding. Furthermore, the observation that no perturbation of soluble CD4-mediated gp120 shedding resulted from dramatic alterations of the V3 sequence (e.g., 308/9/10 RIQ/RPELIPVQ) casts doubt on a necessary role of the V3 loop in the shedding process. We have also demonstrated that a mutant HIV-1 envelope glycoprotein lacking the V3 loop can bind soluble CD4 and shed gp120 as efficiently as the wild-type glycoproteins (73). This conclusion is consistent with the observation that anti-V3 loop neutralizing antibodies do not prevent soluble CD4-induced shedding (50). These data argue against the hypothesis, proposed on the basis of observations made with peptides purportedly mimicking gp120 and CD4 regions (5, 8), that a V3 loop interaction with the CDR3-like region of CD4 triggers gp120-gp41 dissociation, which in turn triggers other events necessary for membrane fusion.

Resistance of naturally occurring HIV-1 variants to the antiviral effects of soluble CD4 (19) may constitute a major barrier to the use of this agent in clinical settings. Therefore, an understanding of the mechanisms underlying resistance may be valuable in designing more effective therapeutic agents based on soluble CD4. Decreases in affinity of the monomeric gp120 glycoprotein for CD4 can result in soluble CD4-resistant, replication-competent HIV-1 variants (46, 66). Some primary patients' isolates that are resistant to soluble CD4, while exhibiting monomeric gp120-CD4 affinities comparable to those of soluble CD4-sensitive viruses, exhibit decreases in the affinity of soluble CD4 for the multimeric envelope glycoprotein complex (11, 48). Thus, decreased affinity of soluble CD4 for the HIV-1 gp120 glycoprotein, which can result either from alterations in the multimeric envelope glycoprotein spike or from changes near the CD4-binding region of the individual gp120 moieties, can lead to soluble CD4-resistant, replication-competent viruses. In this study, a mutant (257 T/G) was identified that exhibited wild-type monomeric gp120-binding affinity for CD4, small decreases in affinity of the multimeric glycoprotein complex for soluble CD4 and in the efficiency of gp120 shedding by soluble CD4, and altered sensitivity to soluble CD4 inhibition of syncytium formation or virus entry. In these characteristics, the 257 T/G mutant resembles some of the primary patients' HIV-1 isolates, although the observed decrease in binding affinity was small for the 257 T/G mutant compared with that reported for the latter variants (48). Some of the amino acid changes associated with soluble CD4 sensitivity in patients' isolates include residues in the second conserved gp120 region, but the exact sequence changes responsible for this phenotype remain to be determined.

Decreased sensitivity to soluble CD4-induced gp120 shedding was observed for four mutants (382 F/L, 420 I/R, 433 A/L, and 438 P/R) that exhibited CD4-binding abilities at least as good as that of the wild-type glycoprotein. This

suggests that high affinity binding of soluble CD4 is necessary, but not sufficient, for the induction of gp120-gp41 dissociation. A second step, presumably involving alteration of the gp120-gp41 interaction, is apparently necessary for efficient gp120 shedding in response to soluble CD4 and is at least partially deficient in the 382 F/L, 420 I/R, 433 A/L, and 438 P/R mutants. Phenylalanine 382, isoleucine 420, alanine 433, and proline 438 are located in a hydrophobic ring formed by the disulfide-linked C3- and C4-conserved gp120 regions (41a). Amino acid changes in this structure, among which the 382 F/L, 420 I/R, 433 A/L, and 438 P/R alterations are included, decrease the affinity of the gp120-gp41 interaction (30). Syncytium-forming and replicative abilities of the envelope glycoproteins are also affected by these amino acid changes, indicating the importance of specific gp120-gp41 interactions in the membrane fusion reaction. Further work will be required to allow any conclusion to be made regarding the identity of gp120-gp41 interactions important for the induction of gp120 shedding by soluble CD4 and for envelope glycoprotein-mediated membrane fusion.

The 382 F/L, 420 I/R, 433 A/L, and 438 P/R mutants, although resistant to soluble CD4-induced gp120 shedding, remained sensitive to the antiviral effects of soluble CD4. Assuming that gp120 shedding on the virions is proportionate to that on the cell surface for a given mutant, this would appear to indicate that, for viruses with a high affinity for CD4, gp120 shedding is not a necessary component of the antiviral activity of soluble CD4. It remains formally possible, however, that these mutant glycoproteins shed more efficiently in the context of the virion spike than on the cell surface. Other amino acid changes in this region may yield additional insight into the contribution of gp120 shedding to soluble CD4 antiviral activity.

ACKNOWLEDGMENTS

We thank Virginia Nixon for manuscript preparation and Amy Emmert for artwork. We thank Marshall Posner for reagents.

M.T. was supported by the Swiss National Science Foundation. J.S. is a Scholar of the Leukemia Society of America and was also supported by the Aaron Diamond Foundation and the National Institutes of Health (AI24755). This work was made possible by a Center for AIDS Research core grant to Dana-Farber Cancer Institute.

REFERENCES

1. **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.
2. **Allan, J., J. Strauss, and D. Buck.** 1990. Enhancement of SIV infection with soluble receptor molecules. Science **247:**1084–1088.
3. **Arthos, J., K. C. Deen, M. Chaikin, J. Fornwald, G. Sathe, Q. Sattentau, P. Clapham, R. Weiss, J. McDougal, C. Piuetropaolo, R. Axel, A. Trumeh, P. Maddon, and R. Sweet.** 1989. Identification of the residues in human CD4 critical for binding of HIV. Cell **57:**469–481.
4. **Ashkenazi, A., L. Presta, S. Marsters, T. Camerato, K. Rosenthal, B. Fendly, and D. Capon.** 1990. Mapping of CD4 binding site for HIV by alanine-scanning mutagenesis. Proc. Natl. Acad. Sci. USA **87:**7150–7154.
5. **Autiero, M., P. Abrescia, M. Dettin, C. DiBello, and J. Guardiola.** 1991. Binding to CD4 of synthetic peptides patterned on the principal neutralizing domain of the HIV-1 envelope protein. Virology **185:**820–828.
6. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauget, C. Axler-Bin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphocyte retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). Science **220:**868–871.
7. **Berger, E., T. 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.
8. **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.
9. **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.
10. **Bowman, M., K. MacFerrin, S. Schreiber, and S. Burakoff.** 1990. Identification and structural analysis of residues in the V1 region of CD4 involved in interaction with human immunodeficiency virus envelope glycoprotein gp120 and class II major histocompatibility complex molecules. Proc. Natl. Acad. Sci. USA **87:**9052–9056.
11. **Brighty, D., M. Rosenberg, I. Chen, and M. Ivey-Hoyle.** 1991. Envelope proteins from clinical studies of human immunodeficiency virus type 1 that are refractory to neutralization by soluble CD4 possess high affinity for the CD4 receptor. Proc. Natl. Acad. Sci. USA **88:**7802–7805.
12. **Brodsky, M., M. Wharton, R. Myers, and D. Littman.** 1990. Analysis of the site in CD4 that binds to the HIV envelope glycoprotein. J. Immunol. **144:**3078–3086.
13. **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.
14. **Callahan, L., M. Phelan, M. Mallinson, and M. Norcross.** 1991. Dextran sulfate blocks antibody binding to the principal neutralizing domain of human immunodeficiency virus type 1 without interfering with gp120-CD4 interactions. J. Virol. **65:**1543–1550.
15. **Choe, H.-R., and J. Sodroski.** 1992. Contribution of charged amino acids in the CDR2 region of CD4 to HIV-1 gp120 binding. J. Acquired Immune Defic. Syndr. **5:**204–210.
16. **Clayton, L., R. Hussey, R. Steinbrich, H. Ramachandran, Y. Husain, and E. Reinherz.** 1988. Substitution of murine for human CD4 residues identifies amino acids critical for HIV gp120 binding. Nature (London) **335:**363–366.
17. **Cordonnier, A., L. Montagnier, and M. Essex.** 1989. Single amino acid changes in HIV envelope affect viral tropism and receptor binding. Nature (London) **340:**571–574.
18. **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.
19. **Daar, E., X. L. Li, T. Moudgil, and D. Ho.** 1990. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc. Natl. Acad. Sci. USA **87:**6574–6578.
20. **Dalgleish, A. G., P. C. L. Beverly, 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.
21. **Deen, K., J. S. McDougal, R. Inacker, G. Folena-Wassterman, J. Arthos, J. Rosenberg, P. Maddon, R. Axel, and R. Sweet.** 1988. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. Nature (London) **331:**82–84.
22. **Fauci, A., A. Macher, D. Longo, H. C. Lane, A. Rook, H. Masur, and E. Gelmann.** 1984. Acquired immunodeficiency syndrome: epidemiologic, clinical, immunologic, and therapeutic considerations. Ann. Intern. Med. **100:**92–106.
23. **Fisher, R., J. Bertonis, W. Meier, V. Johnson, D. Costopoulos, T. Liu, R. Tizard, B. Walker, M. Hirsch, R. Schooley, and R. Flavell.** 1988. HIV infection is blocked *in vitro* by recombinant

soluble CD4. Nature (London) **331**:76–78.

24. **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.
25. **Freed, E., D. 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.
26. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224**:500–503.
27. **Grimaila, R. J., B. A. Fuller, P. D. Rennert, M. B. Nelson, M.-L. Hammarskjöld, 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.
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. **Hussey, R., N. Richardson, M. Kowalski, N. Brown, H. Change, R. Siliciano, T. Dorfman, B. Walker, J. Sodroski, and E. Reinherz.** 1988. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. Nature (London) **331**:78–81.
33. **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.
34. **Ivey-Hoyle, M., J. Culp, M. Chaikin, B. Helling, T. Matthews, R. Sweet, and M. Rosenberg.** 1991. Envelope glycoproteins from biologically diverse isolates of immunodeficiency viruses have widely different affinities for CD4. Proc. Natl. Acad. Sci. USA **88**:512–516.
35. **Jameson, B., P. Rao, L. Kong, B. Hahn, G. Shaw, L. Hood, and S. Kent.** 1988. Location and chemical synthesis of a binding site for HIV-1 on the CD4 protein. Science **240**:1335–1338.
36. **Javaherian, K., A. J. Langlois, C. McDanal, K. L. Ross, L. I. Eckler, C. L. Jellis, A. T. Profy, J. R. Rusche, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1989. Principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein. Proc. Natl. Acad. Sci. USA **86**:6768–6772.
37. **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.
38. **Kowalski, M., L. Bergeron, T. Dorfman, W. Haseltine, and J. Sodroski.** 1991. Attenuation of human immunodeficiency virus type 1 cytopathic effect by a mutation affecting the transmembrane envelope glycoprotein. J. Virol. **65**:281–291.
39. **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.
40. **Landau, N. R., M. Warton, and R. D. Littman.** 1988. The envelope glycoprotein of the human immunodeficiency virus binds to the immunoglobulin-like domain of CD4. Nature (London) **334**:159–161.
41. **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.

41a. **Leonard, C., M. Spellman, L. Riddle, R. Harris, J. Thomas, and T. Gregory.** 1990. Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. J. Biol. Chem. **265**:10373–10382.

42. **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 **323**:725–728.
43. **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.
44. **Matsushita, S., M. Robert-Guroff, J. Rusche, A. Koito, T. Hattori, H. Hoshino, K. Javaherian, K. Takatsuki, and S. Putney.** 1988. Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of the neutralizing epitope. J. Virol. **62**:2107–2114.
45. **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.

45a. **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.

46. **McKeating, J., P. Balfe, P. Clapham, and R. A. Weiss.** 1991. Recombinant CD4-selected human immunodeficiency virus type 1 variants with reduced gp120 affinity for CD4 and increased cell fusion capacity. J. Virol. **65**:4777–4785.
47. **Mizukami, T., T. Fuerst, E. Berger, and B. Moss.** 1988. Binding region for human immunodeficiency virus (HIV) and epitopes for HIV-blocking monoclonal antibodies of the CD4 molecule defined by site-directed mutagenesis. Proc. Natl. Acad. Sci. USA **85**:9273–9277.
48. **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.
49. **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.
50. **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.
51. **Mulligan, M., G. D. Ritter, M. Chaikin, G. Yamshchikov, P. Kumar, B. Hahn, R. Sweet, and R. Compans.** 1992. Human immunodeficiency virus type 2 envelope glycoprotein: differential CD4 interactions of soluble gp120 versus the assembled envelope complex. Virology **187**:233–241.
52. **Olshevsky, U., E. Helseth, C. Furman, J. Li, W. Haseltine, and J. Sodroski.** 1990. Identification of individual HIV-1 gp120 amino acids important for CD4 receptor binding. J. Virol. **64**:5701–5707.
53. **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.
54. **Peterson, A., and B. Seed.** 1988. Genetic analysis of monoclonal

antibodies and HIV binding sites on the human lymphocyte antigen CD4. Cell **54**:65–72.
55. **Posner, M., T. Hideshima, T. Cannon, M. Mukherjee, K. Mayer, and R. Byrn.** 1991. An IgG human monoclonal antibody which reacts with HIV-1 gp120, inhibits virus binding to cells, and neutralizes infection. J. Immunol. **146**:4325–4332.
56. **Putney, S., T. Matthews, W. G. Robey, D. Lynn, M. Robert-Guroff, W. Mueller, A. Langlois, J. Ghrayeb, S. Petteway, K. Weinhold, P. Fischinger, F. Wong-Staal, R. Gallo, and D. Bolognesi.** 1986. HTLV-III/LAV-neutralizing antibodies to an E. coli-produced fragment of the virus envelope. Science **234**: 1392–1395.
57. **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.
58. **Rusche, J. F., K. Javaherian, C. McDanal, J. Petro, D. L. Lynn, R. Grimaila, A. Langlois, R. C. Gallo, L. O. Arthur, P. J. Fischinger, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1988. Antibodies that inhibit fusion of human immunodeficiency virus-infected cells bind a 24-amino-acid sequence of the viral envelope gp120. Proc. Natl. Acad. Sci. USA **85**:3198–3202.
59. **Ryu, S.-E., P. Kwong, A. Truneh, T. Porter, J. Arthos, M. Rosenberg, X. Dai, N. Xuong, R. Axel, R. Sweet, and W. Hendrickson.** 1990. Crystal structure of an HIV-binding recombinant fragment of human CD4. Nature (London) **348**:419–425.
60. **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.
61. **Skinner, M. A., A. J. Langlois, C. B. McDanal, J. S. McDougal, D. P. Bolognesi, and T. J. Matthews.** 1988. Neutralizing antibodies to an immunodominant envelope sequence do not prevent gp120 binding to CD4. J. Virol. **62**:4195–4200.
62. **Smith, D., R. Byrn, S. Marsters, T. Gregory, J. Groopman, and D. Capon.** 1987. Blocking of HIV-1 infectivity by a soluble secreted form of the CD4 antigen. Science **238**:1704–1707.
63. **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.
64. **Stegmann, T., R. Doms, and A. Helenius.** 1989. Protein-mediated membrane fusion. Annu. Rev. Biophys. Biophys. Chem. **18**:187–211.
65. **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.
66. **Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, J. Li, and J. Sodroski.** 1991. Effects of changes in gp120-CD4 binding affinity on human immunodeficiency virus type 1 envelope glycoprotein function and soluble CD4 sensitivity. J. Virol. **65**:5007–5012.
67. **Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, M. Posner, and J. Sodroski.** 1991. Characterization of a discontinuous human immunodeficiency virus type 1 gp120 epitope recognized by a broadly reactive neutralizing human monoclonal antibody. J. Virol. **65**:6188–6193.
68. **Traunecker, A., W. Luke, and K. Karjalainen.** 1988. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. Nature (London) **331**:84–86.
69. **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.
70. **Wang, J., Y. Yan, T. Garrett, J. Liu, D. Rodgers, R. Garlick, G. Tarr, Y. Husain, E. Reinherz, and S. Harrison.** 1990. Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. Nature (London) **348**:411–418.
71. **White, J. M.** 1990. Viral and cellular fusion proteins. Annu. Rev. Physiol. **52**:675–697.
72. **Wiley, D., and J. Skehel.** 1987. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Annu. Rev. Biochem. **56**:365–394.
73. **Wyatt, R., M. Thali, and J. Sodroski.** Unpublished data.