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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

(receptor-induced structural change/human vs. chimpanzee CD4 sequence/vaccinia virus expression vector/AIDS)

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ABSTRACT **We have used a recombinant vaccinia virus vector encoding the envelope glycoprotein of human immunodeficiency virus type 1 to study receptor-induced structural changes related to membrane fusion. A truncated soluble form of human CD4 (sCD4) was found to stimulate dissociation of the external subunit (gp120) from the envelope glycoprotein complex of human immunodeficiency virus type 1 expressed at the cell surface. sCD4 stimulation of gp120 release was time- and concentration-dependent and was associated with specific binding of sCD4 to gp120. Synthetic peptide derivatives corresponding to residues 81–92 of human CD4 (overlapping the complementarity-determining region 3-like region) inhibited cell–cell fusion mediated by the interaction between recombinant vaccinia-encoded CD4 and human immunodeficiency virus envelope glycoprotein. These peptide derivatives also stimulated gp120 release from the envelope glycoprotein complex. An analogous peptide derivative from chimpanzee CD4 (containing a single Glu → Gly substitution at the position corresponding to CD4 residue 87) was considerably less active at inhibition of cell–cell fusion and stimulation of gp120 release, consistent with the known inhibitory effect of this substitution on the ability of membrane-associated CD4 to mediate cell fusion. These results suggest that the sCD4-induced release of gp120 reflects postbinding structural changes in the envelope glycoprotein complex involved in membrane fusion, with the complementarity-determining region 3-like region playing a critical role.**

Human immunodeficiency virus (HIV) infection is initiated by binding of the viral envelope glycoprotein to CD4 molecules on the surface of the target cell (1). The envelope glycoprotein complex consists of an external subunit (gp120) containing the CD4 binding site noncovalently associated with a transmembrane subunit (gp41) that anchors the complex to the virion surface. After binding, direct pH-independent fusion between the virion and plasma membranes ensues (2–6) by a process involving the hydrophobic N terminus of gp41 (7–11). Envelope glycoprotein molecules expressed on the surface of HIV-infected cells can also mediate cell–cell fusion with CD4-positive cells, leading to the formation of multinucleated giant cells (syncytia) (1). The molecular events underlying both virus–cell and cell–cell fusion are poorly understood.

Since the cellular site of HIV fusion and entry is the plasma membrane, we considered the possibility that CD4 binding might trigger structural changes in the gp120–gp41 complex, leading to activation of its fusogenic property and consequent fusion with the plasma membrane. In this report we employ a recombinant vaccinia virus expression system to analyze molecular events underlying membrane fusion. We observe that specific binding of soluble CD4 (sCD4) stimulates dissociation of the gp120–gp41 complex, leading to release of gp120 into the medium (similar to what has been reported with HIV virions and HIV-infected cells; refs. 12–15). Experiments with short synthetic CD4 peptide derivatives provide evidence for the significance of gp120 release for membrane fusion and suggest the involvement of a specific region within the first domain of CD4 previously implicated by biological experiments as being critical for the fusion process.

MATERIALS AND METHODS

Cell Culture and Recombinant Protein Expression. Monolayer cultures of BSC-1 cells and HeLa cells were grown at 37°C in 5% CO_2/95% air in complete medium [minimum essential medium containing 2.5% (vol/vol) fetal bovine serum and antibiotics]. For HIV-1 envelope glycoprotein expression, BSC-1 cells were infected at a multiplicity of infection of 20 with recombinant vaccinia virus vPE16 (16), which encodes the full-length envelope glycoprotein of HIV-1 (strain HTLV-IIIB, BH8 isolate) under control of the vaccinia P7.5 promoter. To obtain human cells expressing recombinant CD4, HeLa cell monolayers were coinfected with two vaccinia virus recombinants, each at a multiplicity of infection of 10. The viruses were vEB-8, which encodes full-length CD4 under control of the bacteriophage T7 promoter (17), and vTF7-3, which encodes the T7 RNA polymerase (18). In the cell fusion and the Western blot analyses, the vaccinia-infected monolayers were treated with trypsin 2–5 hr after infection and then incubated in suspension for 9–14 hr to allow accumulation of the recombinant proteins.

Cell–Cell Fusion Assay. Mixed suspensions were prepared containing an equal number of envelope-expressing and CD4-expressing cells (total density, 1×10^6 cells per ml). Treatment with inhibitors was performed as indicated. Triplicate 0.2-ml samples were dispensed into individual wells of 96-well flat-bottom tissue culture plates (Costar). Syncytia scores (mean of triplicate wells) were determined at various times; an arbitrary value of 4 was assigned to the large syncytia seen in a positive control sample containing a mixture of envelope-expressing cells and CD4-expressing

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Abbreviations: HIV, human immunodeficiency virus; sCD4, soluble CD4; mAb, monoclonal antibody; CDR, complementarity-determining region.

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cells in the absence of inhibitors, and a value of zero was assigned to the single-cell population observed in a negative control sample containing a mixture of envelope-expressing cells and HeLa cells infected with a control vaccinia virus. The experimental samples were arranged randomly on the plate such that their identities were not known during scoring.

gp120 Release Assays. BSC-1 cells expressing recombinant HIV envelope glycoprotein were incubated with the designated agents in complete medium in 5% CO_2/95% air at 37°C, as indicated for each experiment. Envelope glycoprotein polypeptides in the medium and cell lysate fractions were assayed by Western blot or radioimmunoprecipitation analyses; similar results were obtained by the two methods.

For Western blot analysis, BSC-1 cells that had accumulated envelope glycoprotein were treated with 10 μM cycloheximide, and the incubations were continued for 4–6 hr to complete processing of previously synthesized molecules. Cells were washed and incubated at 1×10^6 cells per ml in complete medium containing cycloheximide plus the designated additions (cycloheximide reduced the background of spontaneous gp120 release but did not affect other parameters measured). At the indicated times, the designated fractions were obtained by centrifugation, resolved on SDS/10% polyacrylamide gels, and analyzed on a Western blot (nitrocellulose), using the indicated antiserum followed by ^{125}I-labeled protein A (Amersham). The locations of proteins were visualized by autoradiography.

For radioimmunoprecipitation, BSC-1 monolayers expressing recombinant envelope glycoprotein were labeled between 3 and 8.5 hr after infection in cysteine-free medium supplemented with ^{35}S-labeled L-cysteine (Amersham) at 0.125 mCi/ml (1 Ci = 37 GBq). The cells were chased in complete medium for 12.5 hr, rinsed, and incubated for 2.5 hr in medium containing the indicated concentrations of sCD4. The medium fractions were removed and centrifuged to eliminate debris. Cell lysate fractions were obtained by dissolving the monolayers in phosphate-buffered saline containing 0.5% Nonidet P-40 plus protease inhibitors; insoluble material was removed by centrifugation. For radioimmunoprecipitation, samples were incubated with serum from an HIV-infected patient; immunoprecipitates were collected on protein A-agarose, washed, and resolved on SDS/10% polyacrylamide gels. Labeled proteins were visualized by fluorography (EN^{3}HANCE, New England Nuclear).

Scanning laser densitometry was performed with an LKB Ultroscan XL.

Reagents. sCD4 (residues 1–369) was donated by S. Johnson (Upjohn, Kalamazoo, MI). The anti-CD4 monoclonal antibodies (mAbs) used were OKT4 and OKT4A (Ortho Diagnostics) and anti-Leu3A (Becton Dickinson). Rabbit antisera against recombinant gp120 and gp160 were the generous gifts of R. Willey (National Institute of Allergy and Infectious Diseases).

The CD4 synthetic peptide derivatives used were as follows: 30 [acetyl-$T_bYIC_bE_bVEDQK_{ac}EE$-amide, CD4-(81–92), human sequence], 30* [acetyl-$K_{ac}EEIC_bE_bVEDQT_bY$-amide, scrambled human sequence], 18 [$T_bYIC_bE_bVEDQK_{ac}EE$, CD4-(81–92), human sequence], and 18C [$T_bYIC_bE_bVGDQK_{ac}EE$, CD4-(81–92), chimpanzee sequence]. Subscripts b and ac represent benzyl and acetyl derivatizations, respectively, of the preceding amino acid. Syntheses were performed as described (19).

RESULTS AND DISCUSSION

Cell–Cell Fusion Mediated by Vaccinia-Encoded CD4 and HIV-1 Envelope Glycoprotein. Vaccinia virus vectors have been used to express the envelope glycoproteins of HIV-1 (17, 20–24), HIV-2 (25, 26), and the related simian immunodeficiency virus (27, 28). The fusion specificities of cells expressing these vaccinia-encoded envelope glycoproteins parallel those displayed by the corresponding intact retroviruses, as judged by the blocking activities of anti-CD4 mAbs and sCD4 (17, 22), the lower fusogenic activities of envelope glycoproteins with specific mutations (23, 24, 28) or derived from a nonfusogenic HIV isolate (25), and the restrictions to fusion observed with CD4-bearing cells of various species or cell type (17, 27). Envelope glycoproteins encoded by vaccinia vectors thus provide excellent models for molecular dissection of the fusion process, in the absence of other retrovirus components.

Fig. 1 shows the specificity of cell–cell fusion when BSC-1 cells expressing vaccinia-encoded HIV envelope glycoprotein were mixed with HeLa cells expressing vaccinia-encoded CD4. Fusion was noticeable at 1 hr and progressed over the next 19 hr with the formation of large syncytia; the results at 6 hr are shown. Fusion was dependent on expression of both recombinant proteins, since syncytia did not form when cells infected with a control vaccinia recombinant were substituted for either fusion partner. Fusion specificity was confirmed by blocking with the OKT4A mAb but not the OKT4 mAb and by the inhibitory effect of sCD4 (data not shown).

gp120 Release Induced by sCD4. Guided by the hypothesis that CD4 binding activates the envelope glycoprotein for fusion with the plasma membrane, we studied the effects of sCD4 on envelope glycoprotein structure. In these experiments, the quantity of sCD4 was typically in large molar excess over the envelope glycoprotein. Fig. 2*A* demonstrates that a low level of spontaneous gp120 release occurred in the absence of sCD4, consistent with what has been reported in similar experiments with HIV-infected cells and virions (12, 13, 15). sCD4 stimulated the time-dependent accumulation of gp120 in the medium; in repeated experiments >90% maximal gp120 release occurred within 3–4 hr, as determined by scanning densitometry. The sCD4-stimulated appearance of envelope glycoprotein subunits in the medium was specific for gp120; neither uncleaved gp160 nor gp41 was observed in the medium (Fig. 2*B*). We conclude that sCD4 induced dissociation of the gp120–gp41 complex rather than nonspecific disruption of the membrane. Experiments with mAbs indicated that gp120 release was associated with the specific CD4–gp120 binding interaction. Thus, sCD4 stimulation of gp120 release was inhibited by the anti-CD4 mAbs anti-Leu3A (Fig. 2*C*) and OKT4A (data not shown), which are

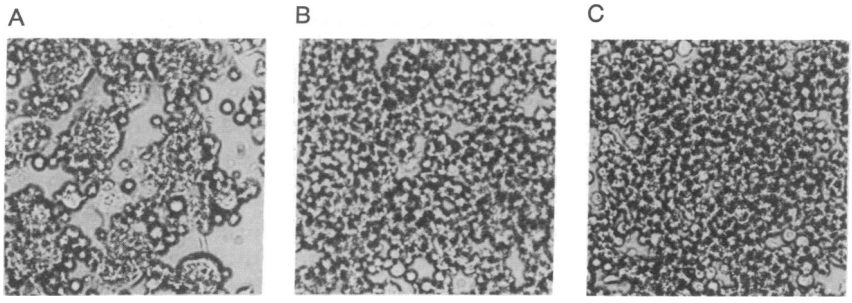

FIG. 1. Specificity of cell–cell fusion mediated by the CD4–HIV envelope glycoprotein interaction in the recombinant vaccinia virus system. The following cell types were mixed: BSC-1 cells infected with vPE16 mixed with HeLa cells infected with vEB-8 plus vTF7-3 (*A*); BSC-1 cells infected with vTF7-3 mixed with HeLa cells infected with vEB-8 plus vTF7-3 (*B*); BSC-1 cells infected with vPE16 mixed with HeLa cells infected with vTF7-3 only (*C*). Photomicrographs were taken 6 hr after cell mixing.

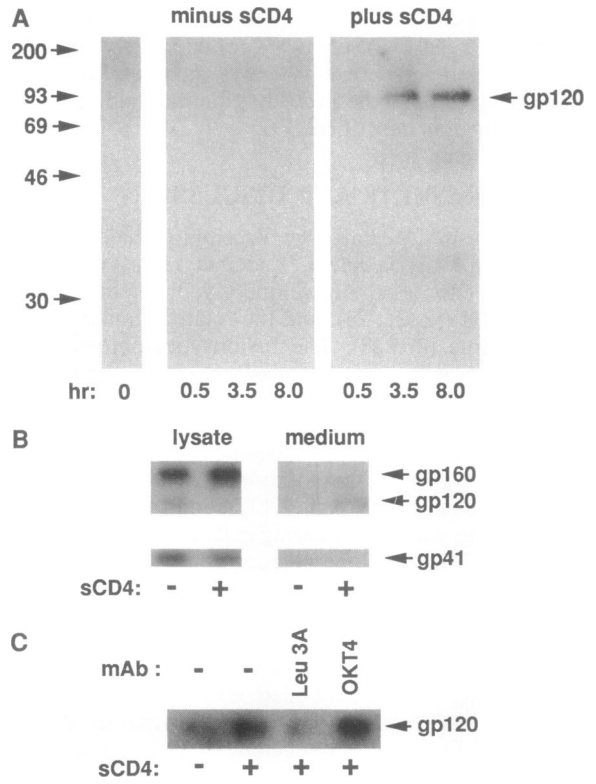

FIG. 2. Stimulation by sCD4 of gp120 release from the HIV envelope glycoprotein complex. BSC-1 cells expressing the HIV envelope glycoprotein were treated as indicated, and the designated fractions were analyzed on Western blots using anti-gp160 antiserum. (*A*) Time course. Cells were incubated for the indicated periods without (minus) or with (plus) 500 nM sCD4, and the medium fractions were analyzed. Molecular mass standards (in kDa) are indicated on the left. (*B*) Envelope glycoprotein subunits in lysate and medium. Cells were incubated with (+) or without (−) 500 nM sCD4 for 4 hr, and the lysate and medium fractions were analyzed. Only the relevant portions of the autoradiogram are shown. The use of anti-gp160 antiserum leads to an overestimate of the relative amount of gp160 compared to gp120. (*C*) Effects of mAbs. Cells were treated for 3 hr without (−) or with (+) 50 nM sCD4 that had been preincubated without (−) or with the indicated mAbs at 500 nM.

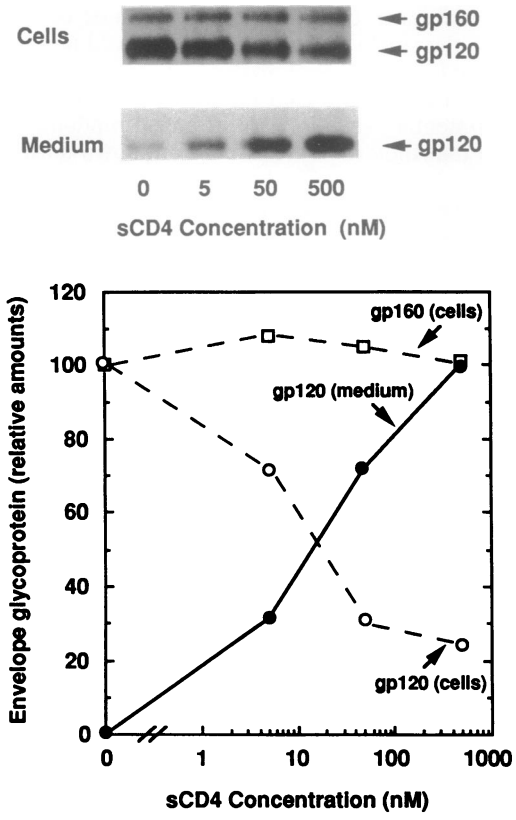

FIG. 3. Concentration dependence of sCD4 stimulation of gp120 release. BSC-1 cells were infected with vPE16 and metabolically labeled, and the cell lysate and medium fractions were analyzed by radioimmunoprecipitation. (*Upper*) Fluorograms show only the relevant envelope glycoprotein bands; each sample represents material derived from the same number of cells with the same exposure time. (*Lower*) The graph shows the corresponding quantitation by scanning densitometry. For gp160 and gp120 in the cell lysates, the values were normalized to the samples incubated without sCD4; for gp120 in the medium, the values were normalized to the samples incubated with 500 nM sCD4, after subtracting the background obtained without sCD4.

potent inhibitors of the CD4–gp120 binding interaction. The control anti-CD4 mAb OKT4, which does not block CD4–gp120 binding, did not inhibit sCD4-induced release of gp120 (Fig. 2*C*).

The dose–response of sCD4-induced dissociation of the gp120–gp41 complex is shown in Fig. 3. At a high sCD4 concentration, most (nearly 80%) of the gp120 was released from the cell into the medium; as noted above, the unprocessed gp160 was not released. The EC_{50} was 12 nM for both depletion of gp120 from the cell and stimulation of gp120 appearance in the medium; however, in repeated experiments, the EC_{50} varied up to 50 nM. Additional experiments (not shown) indicated that sCD4-induced gp120 release was highly temperature-dependent; much less release occurred at 4°C or 23°C compared to 37°C.

The finding that sCD4 stimulated gp120 release in this recombinant system (as well as in HIV-based systems; refs. 12–15) suggests that a related process may occur when the envelope glycoprotein interacts with membrane-associated CD4. Such a structural change potentially could expose the hydrophobic N terminus of gp41, enabling it to interact with the apposing membrane of the target cell. The possibility must also be considered that the fusogenic property of the envelope glycoprotein might be activated by more subtle CD4-induced structural changes than those observed in the gp120 release assay.

Effects of CD4 Synthetic Peptide Derivatives on Cell Fusion and Envelope Glycoprotein Structure. The first domain of CD4 contains regions homologous to the three complementarity-determining regions (CDRs) of immunoglobulin light chain variable regions (designated CDR1, CDR2, and CDR3). Site-directed mutagenesis has emphasized the importance of the CDR2-like region for high-affinity gp120 binding (29–33), though residues in other parts of the first domain may also be involved (34, 35). Particular attention has focused on the CDR3-like region initially implicated by virtue of the ability of synthetic peptides overlapping this region to inhibit HIV infection and associated syncytia formation (19, 36–40). Moreover, based on recent experiments with CD4 mutants (41) and anti-CD4 mAbs (42), it has been proposed that this region plays a critical role in membrane fusion distinct from its involvement in high-affinity gp120 binding.

These findings suggested that synthetic peptide derivatives from the CDR3-like region might provide insight into both the significance of gp120 release for membrane fusion and the regions of the CD4 molecule involved. We focused on short synthetic peptide derivatives representing CD4 residues 81–92 (overlapping the CDR3-like region), which have been analyzed extensively for inhibition of HIV infection and associated syncytia formation (19, 36, 37, 40). Fig. 4 shows the effects of such CD4 peptide derivatives on cell fusion in the vaccinia-based system. Two derivatized versions of human CD4 peptide containing residues 81–92 (designated 30

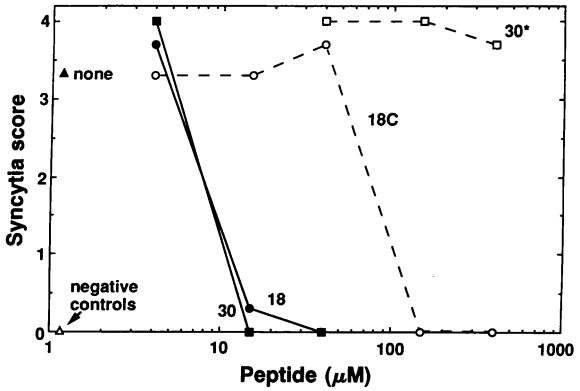

FIG. 4. Effects of CD4 peptide derivatives on cell–cell fusion. BSC-1 cells infected with vPE16 were preincubated for 2 hr at 37°C with the designated peptide derivatives. Peptides: 30, ■; 18, ●; 18C, ○; 30*, □. Cells were then mixed with HeLa cells infected with vEB-8 plus vTF7-3. The positive control with no addition (▲) and the negative controls with one cell type infected with vTF7-3 only (△) are indicated. Syncytia scores were determined at 6 hr.

and 18) blocked cell fusion, with nearly complete inhibition occurring at 15 μM for each peptide. By contrast, a control peptide (designated 30*) containing the identical derivatized and underivatized amino acids as peptide 30 but in a scrambled sequence failed to inhibit at concentrations up to 400 μM. A second control peptide representing residues 23–56 (overlapping the CDR2-like region) also had no effect on membrane fusion at 400 μM (data not shown). These results with the vaccinia-based system parallel those reported for syncytia inhibition in HIV-infected cultures (19, 36, 37, 40).

When the effects of the CD4 peptide derivatives on envelope glycoprotein structure were examined, those peptides that inhibited cell fusion (peptides 30 and 18) were found to stimulate gp120 release (Fig. 5), whereas the control peptides 30* (Fig. 5*A*) and the peptide representing residues 23–56 (data not shown) did not.

Effects of a Single Amino Acid Substitution on the Activity of the CD4 Peptide Derivatives. The chimpanzee CD4 molecule binds gp120 with the same affinity as human CD4 but is markedly deficient in mediating fusion with cells expressing the HIV envelope glycoprotein (41). This cell fusion defect has been attributed to a single amino acid difference within the CDR3-like region, namely glutamic acid in human CD4 vs. glycine in chimpanzee CD4 at residue 87. Thus, the Glu → Gly substitution at position 87 in human CD4 strongly impairs its ability to function in cell fusion without affecting its affinity for gp120, whereas the reciprocal substitution in chimpanzee CD4 renders the molecule capable of supporting cell fusion (41). These findings prompted us to examine the effects of this substitution on the activity of the synthetic CD4 peptide derivatives.

In Fig. 4, the fusion-inhibitory activity of peptide 18 is compared with that of its chimpanzee counterpart (designated peptide 18C), which differs only in the Glu → Gly substitution at the position corresponding to CD4 residue 87. Peptide 18 produced nearly complete fusion inhibition at 15 μM; peptide 18C was ≈10 times less potent. It should be noted that at later times after cell mixing, higher peptide concentrations were required for fusion inhibition, but the diminished potency of peptide 18C was still evident (data not shown).

When effects on envelope glycoprotein structure were examined (Fig. 5*B*), peptide 18 was found to stimulate gp120 release in a dose-dependent fashion; the extent of release at 400 μM peptide was comparable to that achieved with sCD4. By contrast, peptide 18C failed to stimulate gp120 release above background at concentrations up to 400 μM; a small

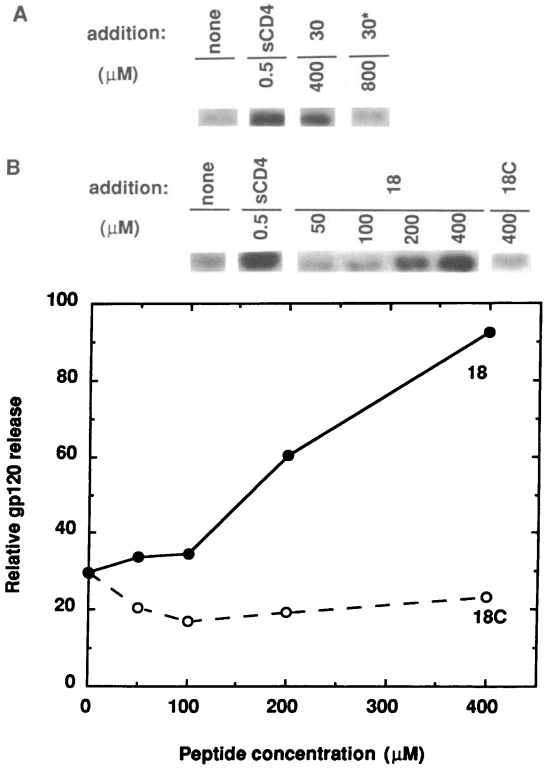

FIG. 5. Stimulation of gp120 release by CD4 synthetic peptide derivatives. The medium fractions from BSC-1 cells expressing the HIV envelope glycoprotein were analyzed on Western blots using anti-gp120 antiserum (only the gp120 bands are shown). (*A*) Cells were treated for 2.5 hr with no addition (none), sCD4, or peptide derivative 30 or 30*, at the indicated concentrations. (*B*) Cells were treated for 3.5 hr with no addition, sCD4, or peptide derivative 18 or 18C, at the indicated concentrations. The graph shows the relative amounts of gp120 determined by scanning densitometry, with the value obtained with sCD4 normalized to 100.

stimulation was observed at 800 μM (data not shown). Thus, a single amino acid substitution known to diminish the cell-fusion activity of membrane-associated CD4 correspondingly diminished the activity of a synthetic peptide derivative from this region for both inhibition of cell fusion and stimulation of gp120 release. These results provide strong evidence for a relationship between gp120 release and membrane fusion. Furthermore, they argue that gp120 release is a consequence of an interaction between CD4 and the envelope glycoprotein that involves the CDR3-like region and that, like cell fusion, is sensitive to a specific amino acid substitution in this region.

Our findings suggest that the CD4 peptide derivatives interact directly with the envelope glycoprotein in the absence of CD4; it is thus reasonable to consider that the fusion-inhibitory activities of the peptides are due to this interaction. We note, however, that the peptide concentrations required for gp120 release are considerably higher than those required for fusion inhibition. Several parameters may contribute to this quantitative discrepancy. (*i*) The HIV-1 envelope glycoprotein exists as an oligomer containing between two and four gp120–gp41 complexes (43–46); the functional unit may in fact be a higher-ordered structure. If most or all gp120–gp41 complexes within a functional unit must be intact for participation in fusion, then release of only a fraction of the gp120 molecules would result in disproportionate fusion impairment. (*ii*) As noted above for CD4, the peptides may induce more subtle changes in envelope glycoprotein structure prior to complete dissociation of the gp120–gp41 complex; at lower peptide concentrations such

changes might impair the fusion activity of envelope glycoprotein molecules without causing gp120 release.

Although the molecular events involved in cell–cell fusion undoubtedly share many features with those involved in virus–cell fusion, recent evidence suggests subtle differences. Both processes appear to involve regions near CDR3, since peptides overlapping CDR3 show inhibitory activity in both syncytia and infectivity assays (19, 36–39). This region has also been implicated in assays of the CD4–gp120 binding interaction, based on mutational analyses (35) and peptide inhibition (19, 40). However, the Glu → Gly substitution at position 87 has markedly different effects on each of these processes. Cell–cell fusion is sensitive to this change, whereas virus infectivity and CD4–gp120 binding are not, judging from studies with mutant CD4 molecules (41) and CD4 peptide derivatives (40). These findings suggest that assays of cell fusion, infectivity, and CD4–gp120 binding involve subtly different molecular interactions that can be revealed by certain amino acid substitutions. The experimental system presented herein involves envelope glycoprotein expressed at the cell surface and thus serves as a model for cell–cell fusion. Accordingly, fusion is sensitive to the Glu → Gly substitution at position 87, as judged by the diminished fusion inhibitory activity of peptide 18C vs. peptide 18. The fact that these peptides show similar differences in gp120 release activity in this system supports the validity of this model for cell fusion studies.

Thus the present findings suggest that the sCD4-induced release of gp120 reflects a CD4-induced structural change in the HIV envelope glycoprotein that is associated with membrane fusion. Furthermore, they provide a structural correlate to the hypothesis, proposed earlier to explain results from biological assays (36–42), that the CDR3-like region functions to activate the envelope glycoprotein for the fusion event.

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1. Klatzman, D. R., McDougal, J. S. & Maddon, P. J. (1990) *Immunodefic. Rev.* **2**, 43–66.
2. Stein, B. S., Gowda, S. D., Lifson, J. D., Penhallow, R. C., Bensch, K. G. & Engleman, E. G. (1987) *Cell* **49**, 659–668.
3. McClure, M. O., Marsh, M. & Weiss, R. A. (1988) *EMBO J.* **7**, 513–518.
4. Maddon, P. J., McDougal, J. S., Clapham, P. R., Dalgleish, A. G., Jamal, S., Weiss, R. & Axel, R. (1988) *Cell* **54**, 865–874.
5. Bedinger, P., Moriarty, A., von Borstel, R. C., II, Donovan, N. J., Steimer, K. S. & Littman, D. R. (1988) *Nature (London)* **334**, 162–165.
6. Sinangil, F., Loyter, A. & Volsky, D. J. (1988) *FEBS Lett.* **239**, 88–92.
7. Gallaher, W. R. (1987) *Cell* **50**, 327–328.
8. Kowalski, M., Potz, J., Basiripour, L., Dorfman, T., Goh, W. C., Terwilliger, E., Dayton, A., Rosen, C., Haseltine, W. & Sodroski, J. (1987) *Science* **237**, 1351–1355.
9. McCune, J. M., Rabin, L. B., Feinberg, M. B., Lieberman, M., Kosek, J. C., Reyes, G. R. & Weissman, I. L. (1988) *Cell* **53**, 55–67.
10. Felser, J. M., Klimkait, T. & Silver, J. (1989) *J. Virol.* **170**, 566–570.
11. Freed, E. O., Myers, D. J. & Risser, R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4650–4654.
12. Kirsh, R., Hart, T. K., Ellens, H., Miller, J., Petteway, S. A., Jr., Lambert, D. M., Leary, J. & Bugleski, P. J. (1990) *AIDS Res. Hum. Retroviruses* **6**, 1209–1212.
13. Moore, J. P., McKeating, J. A., Weiss, R. A. & Sattentau, Q. J. (1990) *Science* **250**, 1139–1142.
14. Moore, J. P., McKeating, J. A., Norton, W. A. & Sattentau, Q. J. (1991) *J. Virol.* **65**, 1133–1140.
15. Hart, T. K., Kirsh, R., Ellens, H., Sweet, R. W., Lambert, D. M., Petteway, S. R., Jr., Leary, J. & Bugleski, P. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2189–2193.
16. Earl, P. L., Hugin, A. W. & Moss, B. (1990) *J. Virol.* **64**, 2448–2451.
17. Ashorn, P. A., Berger, E. A. & Moss, B. (1990) *J. Virol.* **64**, 2149–2156.
18. Fuerst, T. R., Niles, E. G., Studier, F. W. & Moss, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8122–8126.
19. Kalyanaraman, V. S., Rausch, D. M., Osborne, J., Padgett, M., Hwang, K. M., Lifson, J. D. & Eiden, L. E. (1990) *J. Immunol.* **145**, 4072–4078.
20. Chakrabarti, S., Robert-Guroff, M., Wong-Staal, F., Gallo, R. C. & Moss, B. (1986) *Nature (London)* **320**, 535–537.
21. Hu, S.-L., Kosowski, S. G. & Dalrymple, J. M. (1986) *Nature (London)* **320**, 537–540.
22. Lifson, J. D., Feinberg, M. B., Reyes, G. R., Rabin, L., Banapour, B., Chakrabarti, S., Moss, B., Wong-Staal, F., Steimer, K. S. & Engleman, E. G. (1986) *Nature (London)* **323**, 725–728.
23. Kieny, M. P., Lathe, R., Riviere, Y., Dott, K., Schmitt, D., Girard, M., Montagnier, L. & Lecocq, J.-P. (1988) *Protein Eng.* **2**, 219–225.
24. Earl, P. L., Koenig, S. & Moss, B. (1991) *J. Virol.* **65**, 31–41.
25. Mulligan, M. J., Kumar, P., Hui, H., Owens, R. J., Ritter, G. D., Jr., Hahn, B. H. & Compans, R. W. (1990) *AIDS Res. Hum. Retroviruses* **6**, 707–720.
26. Chakrabarti, S., Mizukami, T., Franchini, G. & Moss, B. (1990) *Virology* **178**, 134–142.
27. Koenig, S., Hirsch, V. M., Olmstead, R. A., Powell, D., Maury, W., Rabson, A., Fauci, A. S., Purcell, R. H. & Johnson, P. R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2443–2447.
28. Bosch, M. L., Earl, P. L., Fargnoli, K., Picciafuoco, S., Giombini, F., Wong-Staal, F. & Franchini, G. (1989) *Science* **244**, 694–697.
29. Peterson, A. & Seed, B. (1988) *Cell* **54**, 65–72.
30. Landau, N. R., Warton, M. & Littman, D. R. (1988) *Nature (London)* **334**, 159–162.
31. Clayton, L. K., Hussey, R. E., Steinbrich, R., Ramachandran, H., Husain, Y. & Reinherz, E. L. (1988) *Nature (London)* **335**, 363–366.
32. Mizukami, T., Fuerst, T., Berger, E. A. & Moss, B. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9273–9277.
33. Arthos, J., Deen, K. C., Chaikin, M. A., Fornwald, J. A., Sathe, G., Sattentau, Q. J., Clapham, P. R., Weiss, R. A., McDougal, J. S., Pietropaolo, C., Axel, R., Truneh, A., Maddon, P. J. & Sweet, R. W. (1989) *Cell* **57**, 469–481.
34. Brodsky, M. H., Warton, M., Myers, R. M. & Littman, D. R. (1990) *J. Immunol.* **144**, 3078–3086.
35. Ashkenazi, A., Presta, L. G., Marsters, S. A., Camerato, T. R., Rosenthal, K. A., Fendly, B. M. & Capon, D. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7150–7154.
36. Lifson, J. D., Hwang, K. M., Nara, P. L., Fraser, B., Padgett, M., Dunlop, N. M. & Eiden, L. E. (1988) *Science* **241**, 712–716.
37. Nara, P. L., Hwang, K. M., Rausch, D. M., Lifson, J. D. & Eiden, L. E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7139–7143.
38. Hayashi, Y., Ikuta, K., Fujii, N., Ezawa, K. & Kato, S. (1989) *Arch. Virol.* **105**, 129–135.
39. Shapira-Nahor, O., Golding, H., Vujcic, L. K., Resto-Ruiz, S., Fields, R. L. & Robey, F. A. (1990) *Cell. Immunol.* **128**, 101–117.
40. Lifson, J. D., Rausch, D. M., Kalyanaraman, V. S., Hwang, K. M. & Eiden, L. E. (1991) *AIDS Res. Hum. Retroviruses* **7**, 521–527.
41. Camerini, D. & Seed, B. (1990) *Cell* **60**, 747–754.
42. Truneh, A., Buck, D., Cassatt, D. R., Juszczak, R., Kassis, S., Ryu, S.-E., Healey, D., Sweet, R. & Sattentau, Q. (1991) *J. Biol. Chem.* **266**, 5942–5948.
43. Schwaller, M., Smith, G. E., Skehel, J. J. & Wiley, C. C. (1989) *Virology* **172**, 367–369.
44. Pinter, A., Honnen, W. J., Tilley, S. A., Bona, C., Zaghouani, H., Gorny, M. K. & Zolla-Pazner, S. (1989) *J. Virol.* **63**, 2674–2679.
45. Earl, P. L., Doms, R. W. & Moss, B. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 648–652.
46. Weiss, C. D., Levy, J. A. & White, J. M. (1990) *J. Virol.* **64**, 5674–5677.