A Peptide Inhibitor of Human Immunodeficiency Virus Infection Binds to Novel Human Cell Surface Polypeptides*

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Putative cell surface human immunodeficiency virus (HIV) gp41 receptor proteins of 45 and 80 kDa (p45 and p80, respectively) were identified on human cells using a 17-amino acid peptide, referred to as CS3. In contrast, murine P815 cells expressed a peptide binding protein of 80 kDa only. A segment of 8 amino acids within CS3 contains the minimum sequence able to inhibit binding of radiolabeled CS3 to p80 and p45, as shown by competitive binding studies. Human p45 was purified from CD4+ RH9 cells by CS3 peptide affinity chromatography. Human p80 was partially purified from RH9 cell lysates by size exclusion chromatography followed by SDS-polyacrylamide gel electrophoresis; a rabbit polyclonal antibody was raised against this preparation. Anti-p80 antibody inhibited HIV infection in a dose-dependent manner. The CS3 region of gp41 has been shown previously to be expressed on viral particles and envelope-expressing cells predominantly after conformational changes in the HIV envelope occur due to the interaction of CD4 with gp120. These results, together with those from previous studies, suggest that following the interaction of gp120 with CD4, there may be a second receptor interaction necessary for virus entry/fusion.

The process of cellular entry by the human immunodeficiency virus (HIV) is undoubtedly required multiple events. Binding of the HIV envelope gp120 to CD4 on the cell surface is critical for infection of human CD4+ T lymphocytes (1-4). However, expression of CD4 alone is not sufficient for HIV entry, suggesting a role for additional cellularly encoded molecules (3), and the CD4 molecule is not internalized during HIV entry (5-7). The complexity of HIV entry and its interaction at the cell surface is emphasized by several observations. Notably, specific regions of CD4 not directly involved in gp120 binding may play a role viral entry (8-10). In addition, regions of gp120 that are not part of the CD4 binding site may also play some role in viral entry (11-15). Finally, a putative receptor interaction site at a defined region within the transmembrane glycoprotein, gp41 (16), as well as sequences at both the amino (17) and carboxyl (18, 19) termini of gp41, may be important in viral entry, the formation of syncyta (multinucleated giant cells), or as part of the virus replicative life cycle.

A region defined by amino acids contiguous with the immunodominant domain of gp41, and extending into a region with amphipathic α helix character (20), may define a receptor ligand interaction site necessary for HIV entry (16). A synthetic peptide derived from this region, CS3 (amino acids 576-593 using the HXB2 sequence, Refs. 16, 21, and 22), when coupled to the carrier molecule human serum albumin (HSA, CS3-HSA), bound to specific cell surface proteins and inhibited HIV infection of the human RH9 (CD4+) cell line (16). The importance of the CS3 region of gp41 was also emphasized by studies showing that site-specific mutations leading to nonconservative amino acid changes within the CS3 region of an infectious HIV-1 cDNA render subsequent HIV particles noninfectious (12).

We have suggested previously that stabilization of secondary structure by carrier conjugation may be critical to CS3 activity and a longer peptide or a recombinant form of gp41 may have more potent antiviral properties (16). Recently, it was confirmed that extension of CS3 to 38 amino acids (peptide DP-107) resulted in stable secondary structure, determined by circular dichroism, as compared with CS3 (23). DP-107 was a potent inhibitor of HIV infection and HIV-induced syncytium formation without conjugation of the peptide to a carrier molecule.

One interpretation of these results is that the CS3 containing gp41-derived peptides block the specific interaction of viral gp41 with a cell surface binding protein required for HIV entry or fusion. Alternatively, CS3 containing peptides may interact with a protein(s) that indirectly blocks HIV entry or replication, perhaps by altering the activation state of the cell. In this report, we have characterized the binding of CS3 and CS3-HSA to cell surface proteins of 45 and 80 kDa (p45 and p80, respectively) expressed on the surface of human cell CD4+ cell lines and peripheral blood lymphocytes. Only a p80-like protein was detected on murine p815 cells. Antibody to human p80 blocked HIV-1 infection. We suggest that p45/p80 interact directly with gp41 on the virion. Alternatively, cells may become refractory to HIV infection when cross-linked by antibody to p80 as a result of interaction with multivalent CS3 peptide sequences presented on a carrier molecule (16).

EXPERIMENTAL PROCEDURES

Materials—All peptides were synthesized with a carboxyl-terminal amide by standard solid-phase techniques on methylbenzhydryl-
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ylamine resin. The CS3 peptide (LQARILAVERYLKDQQL) was synthesized by the Peptide Synthesis Unit at the University of Massachusetts, Department of Biochemistry and Molecular Biology and purified by HPLC. A peptide referred to as CKS-17 (LQNRRLDLPLFKEGGL), homologous to a segment from the transmembrane glycoprotein of murine ecotropic virus, was prepared in a similar fashion. Coupling of peptides to carrier human serum albumin (Sigma), and iodomination were performed as described previously (16). Cell lines were maintained in RPMI 1640 containing 10% fetal calf serum (Biofluids Inc., Rockville, MD).

Detection of Peptide-specific Binding Proteins—Cross-linking studies were performed using amidophenylxylopyranosyl APG (APG, Pierce Chemical Co.). Sixty µM 125I-CS3 (10 6 cpm) was mixed with 6 µM APG for 1 h in phosphate-buffered saline (PBS) in the dark. APG was prepared in ethanol at 200 mM and diluted for use as described (24). 125I-CS3-APG (50-500 ng) was incubated with 10 6 cells for the indicated times. The cells were washed three times with cold buffer, then resuspended in 0.5 ml of PBS and exposed, at a distance of 30 cm, to a 300-watt lamp for 15 min. The cells were washed and then resuspended in PBS, containing 0.1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. In some experiments, 10 µg/ml each of aprotinin and leupeptin were added to determine the extent of proteolytic degradation during handling of the lysate. The lysate was precleared by centrifugation at 10,000 g for 5 min, then the supernatant was precipitated using a 5% SDS-PAGE gel for electrophoresis. Dried gels were exposed to x-ray film, and the radioactive band on a 6% SDS-PAGE gel for electrophoresis. Dried gels were exposed to x-ray film overnight.

 Competitive Binding Experiments—For competitive binding studies, unlabeled peptides or CS3-HSA were preincubated with cells for 20-30 min on ice prior to addition of 125I-CS3 for 30 min. The cells were washed, and lysates were prepared and run on SDS-PAGE, as described in the cross-linking experiments above. Inhibition of binding was calculated by comparison of the absorbance (600 nm) of the p45 and p80 signals determined by densitometric scanning of the autoradiograms (Gilford Instruments). The data are displayed as percent inhibition (based upon absorbance) for each competitive agent compared with control.

 Results

 Purification of p80 and Production of Antibody—Cross-linking of 125I-CS3 was performed on 5 x 10 6 RH9 cells, and this lysate was mixed with a lysate from approximately 1 x 10 6 RH9 cells. The lysate (10 ml) was applied in three batches to a S-300 column (250 ml) in PBS containing 0.05% Triton X-100, and the column was run in the same buffer. Fractions were quantified for radioactivity on a γ-counter (Beckman Instruments). The radioactive fractions were pooled, concentrated, and protein was precipitated in 80% ethanol (final concentration). The precipitated protein was electrophoresed by preparative 9% SDS-PAGE; the radioactive band was electroeluted and rerun on a 6% SDS-PAGE gel (Bio-Rad). The gel was exposed to x-ray film, and the radioactive band corresponding to approximately 40 µg of protein was excised, dialyzed against PBS (4 liters x 5 times over 3 days), emulsified by a mortar and pestle, microcentrifuged, and used for immunization of a New Zealand White rabbit. The rabbit was boosted once with similarly prepared material. Immune precipitation was performed using rabbit anti-p80 sera to determine the specificity of the antisera. Lysates of 10 6 cells in 1 ml of lysis buffer were preadsorbed with 30 µl of normal or preimmune rabbit sera for 1 h on ice followed by 100 µl of a 1% solution of protein G-agarose for 30 min (Sigma) and centrifuged to remove the precipitate. Anti-p80 (1-10 µl) was added to the preadsorbed lysate for 1 h on ice followed by addition of 50 µl of protein G-agarose. The pellet was washed in 25 mm phosphate buffer at pH 7.2 containing 0.5 M NaCl, 0.1% Triton X-100, 0.5% Nonidet P-40 and 0.2% SDS. The final pelleted was boiled in SDS-PAGE sample buffer, electrophoresed on a 9% SDS-PAGE gel, then exposed to x-ray film overnight.

 Infection Assay for HIV—Normal preimmune or rabbit anti-p80 sera were added, at the indicated dilutions, to MOLT-4 cells (1.5 x 10 6 cells/ml) for 10 min prior to addition of HIV-1g. Virus was used at a multiplicity of infection of 1.5, determined on HeLa-CD4 cells assayed by end point dilution using p24 antigen capture (Du Pont-Pont-New England Nuclear). After 3 h, the cells were washed four times and resuspended in triplicate wells of 96-well plates in 200 µl of RPMI 1640 containing 10% FCS, and the labeled cells were washed three times in 25 mM phosphate buffer at pH 7.2 containing 0.5 M NaCl, 0.1% Triton X-100, 0.5% Nonidet P-40 and 0.2% SDS. The final pelleted was boiled in SDS-PAGE sample buffer, electrophoresed on a 9% SDS-PAGE gel, then exposed to x-ray film overnight. Control cultures with HIV alone had 60 pg of p24/100 µl at 48 h, whereas this increased to 1600 pg/100 µl by 90 h.

 Results

 Binding of CS3 to Cell Surface Proteins—We used a photoaffinity cross-linking agent, APG (24), to identify any proteins to which radiolabeled CS3 may bind with high affinity. 125I-CS3 cross-linked to two cell surface molecules, with molecular masses of approximately 45 and 73 kDa on nonreducing SDS-PAGE (Fig. 1, A and B). On reducing SDS-PAGE, the 73-kDa protein shifted to 80 kDa and hence was termed p80, whereas the 45-kDa protein migration was unchanged and is referred to as p45 (data not shown). Both p45 and p80 were detected on human cell lines U937 (a monocyte line), THP-1 (a macrophage line), HL-60 (a promyelocyte line), and the CD4+ T cell line RH9.

 In contrast, cross-linking experiments using murine P815 cells (a mastocyteoma) revealed only an 80-kDa binding protein. P815 cells expressing human CD4 are not infectable with HIV-1 (3). Based upon the relative signal of p80 versus p45, it would appear that p80 is 5-10-fold more abundant. However, it is possible that the relative detection of p45 or p80 may be influenced by association with the cross-linking moiety APG, rather than with the peptide CS3. Both p45 and p80 have also been detected on HeLa cells, a human cervical carcinoma, and the human CD4+ cell lines CEM-SS and MOLT-4 (data not shown).

 Comparison of the results of CS3 cross-linking experiments with previous cross-linking studies using a CS3-HSA carrier conjugate (16) suggested that a 45-kDa cellular protein bound
Peptides were added at the indicated concentrations in a preincubation step for 30 min on ice prior to addition of radiolabeled-CS3-APG (first line). Following an additional 30 min on ice and washing, the peptide was cross-linked to its binding proteins by exposure to a 300-watt lamp. Samples were run on a 9% SDS-PAGE, the gel was dried and exposed to x-ray film. A densitometric scan of the autoradiogram was performed, and the data are expressed as percent inhibition of control (lane 1).

| Inhibitor | Concentration | Inhibition |
|-----------|---------------|------------|
| CS3-17    | 80            | 0          |
| CS3-17    | 800           | 0          |
| CS3-17    | 1,600         | 0          |
| CS3      | 80            | 51         |
| CS3      | 800           | 69         |
| CS3      | 1,600         | >95        |
| AVERYLKD  | 100           | 24         |
| AVERYLKD  | 1,000         | 52         |
| CS3-2     | 80            | 0          |
| CS3-2     | 800           | 36         |

**TABLE I**

Percent inhibition of CS3 binding to p45 and p80 by peptides and recombinant gp41

(35%, Fig. 3) blocked binding of 125I-CS3, even when used at a 200-250-fold excess. Lack of inhibition of CS3 binding by CKS-17 suggests that the regions of homology between these two peptides are not critical for binding (see Fig. 3).

An internal 8-mer of CS3 inhibited binding of 125I-CS3 nearly as effectively as unlabeled CS3 (Table I). The ability of a peptide derived from the analogous region of HIV-2 (CS3-2) to inhibit binding was approximately two to three times less efficient than CS3 (36 versus 69% inhibition at 100 ×, respectively). Loss of inhibitory capacity may be ascribed, in part, to two conservative amino acid changes (V to I and R to K) within the internal 8-mer of CS3.

**The Expression of CS3 Binding Domains Increase on Activated Lymphocytes, Cell Lines, and on Cells with Down-regulated CD4**—We examined the expression of CS3 binding sites on human cells cultured in medium alone or medium containing mitogen to learn more about the regulation of CS3 binding sites during lymphocyte activation. Human peripheral blood lymphocytes were activated with PHA or PMA, harvested, and analyzed by flow cytometry for the amount of CS3 binding after 48 h of culture, using CS3-HSA-FITC as described previously (16). PBMC expressed two populations of CS3 binding cells when freshly isolated or after 48-h culture in medium. One population of cells (<5%) was defined by a high density of CS3 binding as compared with the majority of the cells (Fig. 4A). Following activation with either PHA or PMA, increased expression of CS3 binding was observed (Fig 4). Most cells showed increased CS3 binding after treatment with PMA (>90%, Fig. 4C), whereas PHA activation increased CS3 binding on approximately 40% of the cells (Fig. 4B). PMA treatment of human lymphocytes results in loss of CD4 expression (25-29). However, we observed that CS3 binding
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Control

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Fig. 4. Expression of CS3 binding domains on resting and activated PBMC shown in one of three similar experiments. Analysis for CS3 binding domains using CS3-HSA-FITC has been described previously (16). Following culture in medium alone (A), with PHA (B) or PMA (C), flow cytometry was performed using 1 µg of CS3-HSA-FITC/million cells (filled histogram). As a control (open histogram), an identical quantity of HSA-FITC or an irrelevant peptide conjugated to HSA-FITC (see Ref. 16) was used.

increased following treatment with PMA. To investigate this more closely, we used human CD4+ cell lines to examine any linkage between CD4 expression and that of the CS3 binding sites.

Several CD4+ cell lines (RH9, MOLT-4, and MT-4) were examined for expression of CS3 binding following activation. Similar results were observed on all three cell lines in three independent experiments with each cell line and are represented by results with RH9 cells (Fig. 5). Comparison of the expression of CD4 and CS3 binding at 24-h intervals following PMA treatment demonstrated rapid disappearance (>90%) of CD4 (Fig. 5A), as previously described (25–29). In contrast, binding of CS3-HSA-FITC showed an overall increase at the same time that CD4 expression decreased (Fig. 5, B versus A, respectively).

Purification of CS3-binding Proteins—Using both CKS-17 and CS3 immobilized onto a resin, we were able to detect the presence of binding proteins only with the CS3 affinity gel (Fig. 6A). Four radiolabeled RH9 cell proteins eluted from the CS3 affinity column with free CS3 peptide. One protein of approximately 200 kDa has only been observed in one of three experiments performed and was not observed in preparative scale purification (Fig. 6B). A 35-kDa protein was also...
observed in affinity purification (Fig. 6B), but only in 2 out of 12 cross-linking experiments. It is currently unknown whether the 35-kDa protein is a degradation product of p45 or part of what could be a heteromultimeric complex. The remaining two proteins correspond to p80 and p45 which were also detected by CS3 cross-linking experiments (Fig. 1). After CS3 peptide elution of the CS3 affinity column (Fig. 6A, lane 3), washing the column with pH 3.0 glycine failed to elute any additional detectable protein (lane 4). In contrast, no detectable proteins could be eluted from the CKS-17 column with either CS3 or acid glycine (Fig. 6A, lanes 1 and 2, respectively).

Larger scale preparative (10⁶ RH9 cells) purification of binding proteins on a 10-ml CS3 affinity column resulted in isolation of predominately p45 which was eluted in early fractions by acid glycine (Fig. 6B). Preparative isolation of p45 has been achieved using RH9 and CEM-SS cell lines in a total of 10 experiments and p45 has been stained using Coomassie Blue, Ponceau S, and silver stain. Sequencing attempts have been unsuccessful due to a blocked NH₂ terminus.

P80 was partially purified by S-300 chromatography after cross-linking to ¹²⁵I-CS3 (Fig. 7). Pooled radioactive fractions in the 200-250-kDa molecular size range were concentrated on an Amicon concentrator with a 30-kDa cut-off membrane, then purified by SDS-PAGE and used for immunization.

Anti-p80 Antibody Inhibits HIV-1 Infection—Rabbit antibody specific for p80 immune-precipitated a single protein band from surface iodinated RH9 cells (Fig. 8). The cellular lysates were precleared with preimmune sera and protein A-agarose prior to the addition of anti-p80.

Next we tested nonimmune (preimmune sera, sera from a litter mate, and pooled rabbit sera (Pelfreeze)) or rabbit anti-p80 antibody for inhibition of HIV infection by incubating sera with MOLT-4 cells followed by the addition of HIV-1 (Fig. 9). Antigen capture (HIV p24) assay performed at 48 and 90 h showed that p24 levels increased over 26-fold in this 32-h period. At 90 h we observed somewhat less inhibition of HIV infection by anti-p80 antibody as compared with 48 h, but the amounts of p24 in control cultures (maximum p24) at 90 h were at the highest concentration on the standard curve (400 pg/well) and may be underestimations. Greater than 95% inhibition of HIV-1 infection was observed at anti-p80 serum dilutions of 1:40, whereas normal and preimmune sera inhibited by 10% or less. Fifty percent inhibition of HIV-1 infection was observed at dilutions of anti-p80 between 1:800 and 1:1000 in all three experiments. Primary rabbit anti-p80 antisera taken 3 weeks after the first immunization had only weak neutralizing activity for HIV-1 infection (50% inhibition
The studies described herein reveal that the gp41-derived peptide CS3 and the carrier conjugate CS3-HSA interact with human cell surface molecules of approximately 45 and 80 kDa. p45 and p80 are distributed on cells of both hematopoietic and nonhematopoietic origin (HeLa cells, data not shown). Conjugation of CS3 to a carrier molecule, HSA, significantly enhanced its interaction with p45 and p80. Stabilization of peptide secondary structure by conjugation to the carrier molecule or multiple receptor interactions by CS3-HSA molecules may contribute to the observed higher binding avidity of CS3-HSA compared with CS3 (16). Binding of CS3 was not dependent upon CD4 expression. Treatment of PBMC, RH9, or other human CD4+ cell lines with phospholipid esters resulted in loss of CD4, whereas the expression of CS3 binding sites increased showing that CS3-binding proteins are not linked to CD4.

Competition for binding between radiolabeled CS3 and unlabeled peptides suggests that an internal 8-mer (AVRNLKD) contains critical binding residues. This conclusion is supported by the inability of CKS-17 to inhibit binding. CKS-17 has only 2 amino acids in common within the 8-mer sequence. However, amino acids outside this internal 8-mer of CS3 may also contribute to binding specificity by stabilizing secondary structure. Comparison of the ability of the 8-mer to inhibit binding of [35S]CS3 versus inhibition by the CS3-2 (HIV-2-derived) peptide suggests that even conservative changes in the 8-mer sequence may affect binding. The control peptide used in these studies, CKS-17, is a consensus sequence peptide derived by comparison of similar amphipathic helical sequences of the transmembrane glycoproteins of several retroviruses (30). CKS-17, which contains a leucine repeat motif similar to CS3, is unable to block the binding of CS3 to p45 or p80 when used alone (Table I) or in conjugated form (16), suggesting that binding to p45 and p80 is not a general property of amphipathic peptides. In addition, CKS-17 affinity chromatography failed to demonstrate the presence of any specific binding proteins on human cells (Fig. 6A).

The complete characterization of p45 and p80 is in progress, but preliminary results suggest several possibilities. Purification of predominately p45 by affinity chromatography suggests that it likely contains a high affinity binding site, whereas size exclusion chromatography shows that p45 and p80 migrate at a size consistent with a multimeric complex. Together, the results suggest that the complex of p45 and p80 likely dissociates during conditions used for affinity chromatography and immune precipitation by anti-p80. Alternatively, p45 and p80 may be independent molecules that form weak associations following detergent treatment of RH9 cells and only appear to run as a complex by size exclusion chromatography. Probing with monoclonal antibodies will be necessary to determine the relationship between p45 and p80. Another protein of approximately 35 kDa appears in the affinity isolation of p45 (see Fig. 6). The identity of this protein is not known, and it does not appear in cross-linking experiments, but efforts to sequence this protein and prepare specific antibody are in progress.

CS3 and CKS-17 carrier conjugates have been shown to have antiproliferative properties. Peptide carrier conjugates of CS3 or CKS-17 inhibit mitogen stimulation of primary human lymphocytes, but only at high concentrations of peptide (>29 μg/ml, approximately 10 μM) (30-32). The inability of CKS-17 to inhibit binding of CS3 and the inability to detect any protein binding to CKS-17 affinity columns suggest that the antiproliferative properties of these peptide carrier conjugates may be independent of cell surface binding.

The possibility that CS3 binding has an effect on cell activation or virus life cycle, other than at the point of entry, has been addressed. Continuous 48-h treatment of RH9 cells, chronically infected with HIV-1, or HIV-1muc, with 10 μg/ml CS3-HSA had no effect on the production of HIV (data not shown). In contrast, RH9 cells were exposed to CS3-HSA for only 3 h in experiments to inhibit HIV infection (16). In addition, CS3 carrier conjugates failed to inhibit the proliferation of RH9 or MOLT-4 cells or mitogen-induced proliferation of human PBMC, measured by incorporation of [3H] thymidine, when used under the same conditions described for their antiviral effect (16). This result is consistent with previous reports (21, 22, 31). Although together these results support the notion that CS3/receptor interaction does not alter cell activation under the conditions used, we cannot exclude this possibility until further studies are performed.

A role for p80 in HIV infection was indirectly shown by the ability of anti-p80 to block HIV infection. One interpretation of this result is that HIV gp41 interacts with p80, and antibody prevents this interaction. However, inhibition of HIV infection by a polyclonal antibody must be confirmed by the use of monoclonal antibodies. We cannot exclude the possibility that (i) the antisera may react with some other cell surface antigen(s) in addition to p80, or (ii) inhibition of infection is an indirect effect of the interaction of p80 with antibody. Anti-p80 did not react with HIV-1 on commercial Western blots (BioTech Research Labs, Rockville, MD) ruling out the possibility that inhibition of virus production could be caused by cross-reactivity with viral envelope proteins (data not shown).

Recent results by Wild et al. (23) confirm and extend our previous report (16) regarding the importance of the CS3 region of gp41 as an inhibitor of HIV infection. These investigators showed that stabilization of secondary structure through the use of a longer peptide is essential for anti-viral activity. It was suggested that the gp41 peptide used in these studies, DP-107, may affect the HIV virion directly. However, treatment of virus with DP-107 had no effect on the infectious titer of subsequently pelleted virus (23). Although this possibility would require significant amounts of free energy to either displace gp120 or interrupt the gp41 multimeric structure on the viral particle, it cannot be formally excluded. The more potent anti-viral activity exhibited by a DP-107 dimer (25) supports our conclusions regarding the multivalency of CS3-HSA as an important factor for anti-viral activity (16). Furthermore, stabilization of CS3 secondary structure by carrier conjugation may also explain the biological activity of CS3-HSA as opposed to CS3, since conjugation of peptides to carrier molecules has been shown to stabilize peptide secondary structure (33).

The CS3-containing region of gp41 (amino acids 579-613) is cryptic until sCD4 binds to gp120 (34). Sheding of gp120 has also been reported following binding of sCD4 to gp120 (35-37), but the putative role of gp120 shedding in entry is unknown and may be isolate-dependent. Inhibition of infection by primary isolates of HIV-1 by sCD4 requires approximately 100-1000 times more sCD4 than does neutralization of isolates passaged in cell lines (38, 39). However, no changes in the affinity between gp120 and CD4 of a similar magnitude have been noted upon comparison of primary and passaged isolates (39-42). One explanation for these observations could lie in the relative role of CD4 binding and interaction of virus

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with other cell surface proteins required for entry. CD4-induced conformational changes in the HIV envelope leading to interaction with non-CD4 cellular proteins may "hinge" upon sequences within the V3 loop, where resistance to sCD4 has been mapped recently (39).

If gp41 binding to cellular receptors is a requirement for HIV entry, then we would expect that this event may occur rapidly following a conformational change in envelope (gp120/gp41) induced by CD4 binding (34). Murine P815 cells expressing human CD4 bind HIV, but no infection was observed (3). Murine cells are, however, capable of expressing HIV following transfection of appropriate proviral DNA (43). Furthermore, CD4 transgenic mice cannot be infected with HIV nor can CD4- cells derived from these mice (44). Finally, a recent report using murine-human hybrid cells has also concluded that human cells contribute a component(s), in addition to CD4, essential for virus entry/fusion (45). Together with the detection of p80-like proteins in human and murine cells, these results suggest that restricted distribution of p45 to human cells or functional differences between human and murine p80 may make murine cells refractory to HIV entry.

We cannot rule out the possibility that other cell surface proteins are involved in HIV infection. However, no other candidate molecules have been firmly established with the exception of a galactosyl ceramide found in neural cells (46).

In conclusion, we have identified at least two non-CD4 candidate molecules that may be involved in HIV-1 entry, following the interaction of CD4 and gp120. The evidence suggests that p45/p80 may form a multimeric complex. Antibody to one of these proteins, p80, inhibited HIV-1 infection. Studies are in progress to fully address the role of p45 and p80 in HIV entry and to clone the genes which encode p80 and p45.

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