A synthetic peptide inhibitor of human immunodeficiency virus replication: Correlation between solution structure and viral inhibition

(leucine zipper/gp41/envelope multimerization)

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ABSTRACT A peptide designated DP-107 was synthesized containing amino acid residues 558–595 of the envelope glycoprotein gp160 of human immunodeficiency virus type 1 strain LAI (HIV-1_{LAI}). Algorithms for secondary structure have predicted that this region of the envelope transmembrane protein should form an extended α -helix. Consistent with this prediction, analysis by circular dichroism (CD) indicated that, under physiological conditions, DP-107 is $\approx 85\%$ helical. The high degree of stable secondary structure in a synthetic peptide of this size suggests self-association typical of a coiled coil or leucine zipper. In biological assays, the peptide efficiently blocked virus-mediated cell–cell fusion processes as well as infection of peripheral blood mononuclear cells by both prototypic and primary isolates of HIV-1. A single amino acid substitution in the peptide greatly destabilized its solution structure as measured by CD and abrogated its antiviral activity. An analogue containing a terminal cysteine was oxidized to form a dimer, and this modification lowered the dose required for antiviral effect from 5 to about 1 $\mu\text{g}/\text{ml}$. These results suggest that both oligomerization and ordered structure are necessary for biological activity. They provide insights also into the role of this region in HIV infection and the potential for development of a new class of antiviral agents.

Of the anti-human immunodeficiency virus (HIV) treatment strategies tested to date, none is curative and only certain inhibitors of the viral reverse transcriptase (RT) have demonstrated clinical benefit (1). As a result, the search for new, more efficacious drugs and/or other sites on the virus against which to target antiviral therapy continues at an urgent pace. In this report we describe a synthetic peptide which appears to possess potent antiviral activity *in vitro* and may represent a lead to a new class of antiretroviral agents. The peptide sequence is based on a highly conserved region in the transmembrane (TM) protein which was predicted by Gallaher *et al.* (2) to form an extended amphipathic α -helix with structural analogues in the TM proteins of several fusogenic viruses, such as influenza virus and other retroviruses. The function of the site is not known but may be related to multimerization of the envelope glycoprotein.

Our interest in the TM protein region described above was heightened by reports that its primary sequence is strongly predictive of an extended amphipathic α -helix (2) and contains a “leucine zipper” repeat (3). O’Shea *et al.* (4) have demonstrated that the latter type of structural motif can be modeled using synthetic peptides. We reasoned that if this region of gp41 exhibited a similar type of structure (specifically, subunit oligomerization to form coiled coils) we might successfully model this process with synthetic peptides. If so, then these models could be used to study the relationship of

secondary structure to several important aspects of viral replication, including multimerization and the effect of mutations within gp41 on this process. The results of such experiments are reported here and suggest a highly ordered and stable structure for this region of the HIV-1 TM protein. In addition, these peptides exhibited strong antiviral activity, blocking both infection by cell-free virus as well as formation of syncytia mediated by infected cells at peptide concentrations as low as 1 $\mu\text{g}/\text{ml}$. These inhibitory activities were unexpected, and, at this time, the mechanisms involved are not understood, although several possibilities are discussed.

MATERIALS AND METHODS

Peptide Synthesis. Peptides were synthesized using “Fast Moc” chemistry on an Applied Biosystems model 431A peptide synthesizer. Amidated peptides were prepared using Rink resin (Advanced ChemTech), whereas peptides containing free C termini were synthesized on Wang (*p*-alkoxybenzyl alcohol) resin (Bachem). First residues were double coupled to the appropriate resin and subsequent residues were single coupled. Each coupling step was followed by acetic anhydride capping. Peptides were cleaved from the resin by treatment with trifluoroacetic acid (10 ml), water (0.5 ml), thioanisole (0.5 ml), ethanedithiol (0.25 ml), and crystalline phenol (0.75 g). Purification was carried out by reverse-phase HPLC. Samples (≈ 50 mg) of crude peptide were chromatographed on a Waters Delta Pak C₁₈ column (19 mm $\times$ 30 cm, 15- μm spherical resin) with a linear gradient: water/acetonitrile in 0.1% trifluoroacetic acid. Lyophilized peptides were stored desiccated and peptide solutions were made in water at about 5 mg/ml. Peptides stored in solution were stable for an extended period of time (>6 weeks) at 4°C and could be frozen and thawed repeatedly with little apparent effect on biological activity. Electrospray mass spectrometry yielded the following results: DP-107, m/z 4526.71 (calculated 4526.31); DP-121, m/z 4510.75 (calculated 4510.27); DP-116, m/z 2057.32 (calculated 2056.44); DP-125, m/z 4743.46 (calculated 4743.55); DP-31, m/z 2479.35 (calculated 2480.93).

Circular Dichroism. CD spectra were measured in 10 mM sodium phosphate/150 mM sodium chloride, pH 7.0, with peptides at ≈ 10 μM in a 1-cm-pathlength cell on a Jobin/Yvon Autodichrograph Mark V CD spectrometer. Peptide concentrations were determined from A₂₈₀ (5).

Virus. HIV-1_{LAI} was obtained from R. Gallo (6) and propagated in CEM cells cultured in RPMI 1640 containing 10% fetal bovine serum. Supernatant from the infected CEM

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Abbreviations: HIV, human immunodeficiency virus; RT, reverse transcriptase; PBMC, peripheral blood mononuclear cell; TM, transmembrane; TCID, tissue culture infectious dose.

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cells was passed through a 0.2- μ m filter and the infectious titer was estimated in a microinfectivity assay using the AA5 cell line to support virus replication. For this purpose, 25 μ l of serially diluted virus was added to 75 μ l of AA5 cells at a concentration of 2×10^5 per ml in a 96-well microtiter plate. Each virus dilution was tested in triplicate. Cells were cultured for 8 days by addition of fresh medium every other day. On day 8 postinfection, supernatant samples were tested for virus replication as evidenced by RT activity. The 50% tissue culture infectious dose (TCID₅₀) was calculated according to the formula of Reed *et al.* (7). The titer of the HIV-1_{LAI} stock used for these studies, as measured on the AA5 cell line, was $\approx 10^7$ TCID₅₀/ml. The two primary isolates were obtained from peripheral blood mononuclear cells (PBMCs) of two infected donors, one from Brazil (HIV-1_{Br3}) and the other from Trinidad (HIV-1_{Q2275}), by cocultivation with phytohemagglutinin-stimulated normal donor PBMCs in RPMI 1640 containing interleukin 2. The infectious titers of the primary virus stocks were estimated by titration on phytohemagglutinin-stimulated normal human PBMCs in a 96-well microtiter plate, again by using RT activity released to the supernatant as evidence of successful infection. The infectious titer of both these isolates was estimated to be $\approx 10^3$ TCID₁₀₀/ml.

Cell Fusion Assay. MOLT-4 cells ($\approx 7 \times 10^4$) were incubated with CEM cells (10^4) chronically infected with HIV-1_{LAI} in 96-well plates (half-area cluster plates; Costar) in 100 μ l of culture medium, as described (8). Peptide inhibitors were added in 10 μ l and the cell mixtures were incubated for 24 hr at 37°C. At that time, multinucleated giant cells were estimated by microscopic examination at $\times 40$ magnification, which allowed visualization of the entire well in a single field.

Virus Ultracentrifugation. To remove non-virus-associated peptides or soluble CD4, a 0.4-ml sample of treated virus was layered onto 0.15 ml of phosphate-buffered saline containing 5% sucrose in a Beckman SW50 minitube (total capacity about 0.6 ml) and centrifuged at 35,000 rpm for 60 min at 4°C. The supernatant was aspirated and the virus pellet was resuspended in RPMI 1640 containing 10% fetal bovine serum.

RT Assay. The RT microassay was adapted from refs. 9 and 10. Supernatants from virus/cell cultures were made 1% (vol/vol) in Triton X-100. A 10- μ l sample of supernatant was added to 50 μ l of RT cocktail in a 96-well U-bottom microtiter plate and incubated at 37°C for 90 min. The cocktail contained 75 mM KCl, 2 mM dithiothreitol, 5 mM MgCl₂, poly(A) (5

μ g/ml; Pharmacia cat. no. 27-4110-01), oligo(dT) (0.25 unit/ml; Pharmacia cat. no. 27-7858-01), 0.05% Nonidet P-40, 50 mM Tris-HCl (pH 7.8), 0.5 μ M nonradioactive dTTP, and [α -³²P]dTTP (10 μ Ci/ml; Amersham cat. no. PB.10167; 1 Ci = 37 GBq). After incubation, 40 μ l of reaction mixture was applied to a Schleicher & Schuell NA45 membrane (or Whatman DE81 paper) saturated in 2 $\times$ SSC (0.3 M NaCl/30 mM sodium citrate, pH 7) in a Schleicher & Schuell Minifold over one sheet of GB003 filter paper. Each well of the minifold was washed four times with 200 μ l of 2 $\times$ SSC. The membrane was removed and washed twice in a Pyrex dish with an excess of 2 $\times$ SSC. Finally the membrane was drained on absorbent paper, placed on Whatman no. 3 paper, covered with SaranWrap, and exposed to film overnight.

RESULTS

Evidence for Structure of Synthetic Peptide in Solution. DP-107 is a 38-amino acid peptide corresponding to residues 558–595 of the HIV-1 TM protein (Fig. 1). The primary sequence of this region is strongly predictive of α -helical secondary structure and contains a "leucine zipper" repeat (2, 3). The N terminus of the peptide was acetylated and the C terminus amidated to reduce unnatural charge effects at those positions. DP-107 and the other peptides used in this study (Fig. 1) were purified by reverse-phase HPLC, and, in each case, the purified peptides gave a single symmetrical peak by analytical HPLC. The identity of each peptide was confirmed by electrospray mass spectrometry.

The ultraviolet CD analyses of DP-107 (summarized in Fig. 2) suggest a considerable amount of secondary structure for the peptide under physiologic conditions. The double minima at 222 and 208 nm are characteristic of α helices, and mean molar ellipticity values ($[\theta]_{222}$) of $-32,000$ at 0°C (Fig. 2A) and $-27,000$ at 37°C (Fig. 2B) indicate that the peptide is approximately 100% and 85% folded at these temperatures (11, 12). The stability of the observed structure is illustrated by the thermal melt data (Fig. 2C). For example, at 10 μ M DP-107, the midpoint of the melting curve (T_m) was approximately 72°C. The concentration dependence of the T_m for DP-107 (Fig. 2C) is characteristic of leucine zipper-type structures and is indicative of the stabilization of secondary structural elements by self-association (4). Oligomerization of DP-107 in solution to form dimers and tetramers is also suggested by preliminary sedimentation equilibrium studies (W. Stafford and C.W., unpublished work). The results shown in Fig. 2

FIG. 1. Schematic representation of HIV-1_{LAI} transmembrane protein gp41 and sequences of the peptides studied. In DP-107, DP-121, and DP-125 the leucine or isoleucine heptad repeat units are underlined. DP-107, DP-121, and DP-125 are acetylated at the N terminus and amidated at the C terminus. DP-116 (identical to the CS3 peptide) is amidated at the C terminus and has a free N terminus. DP-31 is neither acetylated nor amidated. Amino acid residues are numbered according to ref. 25.

FIG. 2. CD spectra are shown for 10 μ M DP-107 ($\circ$), DP-121 ($\square$), and DP-116 ($\bullet$) at 0°C (A) and 37°C (B). The midpoint of the temperature dependence (T_m) of the CD signal for DP-107 is concentration-dependent (C). T_m corresponds to the maximum of the first derivative of the CD melt curve. CD spectra were obtained in 10 mM sodium phosphate/150 mM sodium chloride at pH 7.0.

tend to support the predictions (2, 3) that the region of gp41 corresponding to DP-107 contains a leucine zipper-like (coiled coil) motif which may play a role in envelope oligomerization. This type of structure can be described as a homodimer formed by the specific (and often parallel) association of two α -helices. This interaction is characterized by the alignment of the hydrophobic faces of the helices. The unusually stable secondary structure exhibited by peptides involved in these types of systems is due to these higher-order interactions. Preliminary analysis of the solution structure of DP-107 by multidimensional NMR spectroscopy indicates a large number of sequential NH–NH₂ crosspeaks in the two-dimensional nuclear Overhauser enhancement spectra (C.W., unpublished work), which is consistent with the CD evidence that under physiologic conditions, the peptide exhibits significant α -helical secondary structure.

The CD spectra of two other synthetic peptides are also shown in Fig. 2. One of these, DP-121, is identical to DP-107 but with the isoleucine at position 578 replaced by a proline residue. The other peptide, DP-116, is a 17-mer and overlaps the C terminus of DP-107. This peptide was synthesized to contain the same amino acids and blocking groups as CS3, a peptide described by Qureshi *et al.* (13) and reported to exhibit antiviral activity when coupled to albumin. The CD spectra observed for these two peptides indicate that both exist in random coil conformations at 37°C, in direct contrast to the results obtained for DP-107. This outcome was expected for the proline-substituted analogue, DP-121, because the proline would tend both to break helix formation and to disrupt hydrophobic interactions thought to stabilize coiled coil structures.

Peptide Inhibition of Infected Cell-Induced Syncytium Formation. The initial screen for antiviral activity of the peptides shown in Fig. 1 was for blockade of syncytium formation induced by overnight cocultivation of uninfected MOLT-4 cells with chronically infected (HIV-1_{LAI}) CEM cells. The results of three such experiments are shown in Table 1. In the first of these, serial concentrations of peptide between 50 and 1.5 μ g/ml were tested for blockade of the cell fusion process. DP-107 afforded complete protection down to a concentration of 6 μ g/ml. The overlapping 17-mer, DP-116, which is analogous to the previously described CS3 (13), exhibited no evidence of antifusogenic activity even at 50 μ g/ml. This observation is in agreement with that of Qureshi *et al.* (13), who found antiviral activity for CS3 only after it was conjugated to albumin. A second peptide, DP-31, representing an overlapping immunodominant site (14, 15), also failed to show inhibitory activity (Table 1).

The inhibitory activity of DP-107 did not appear to be related to cytotoxic or cytostatic effects, since in other studies (data not shown) CEM cells grown in the presence of

DP-107 at 50 μ g/ml (the highest concentration tested) for 3 days (with fresh peptide added each day) displayed the same viability and growth rate as control cultures. We also found that DP-107 blocked fusion mediated by three other prototypic HIV-1 isolates: MN, RF, and SF2 (data not shown).

The concentration dependence of the CD spectra suggests that the structure of DP-107 is stabilized by peptide self-association. In similar studies, O'Shea *et al.* (4) reported that disulfide bridging of a peptide of the leucine zipper domain in the GCN4 protein (a yeast transcriptional regulatory factor) to form covalently bonded homodimers stabilized the coiled coil structure. Following similar reasoning, we sought to determine whether the limiting effective concentration for cell fusion blockade by DP-107 might in part be related to the concentration dependence of peptide self-association. To test this possibility we synthesized a DP-107 analogue with a cysteine-containing "tail" which after purification could be air-oxidized to yield a homodimer. The resulting peptide, DP-125, exhibited approximately twice the apparent molecular weight of DP-107 in SDS/PAGE under nonreducing conditions, suggesting that a covalently bonded homodimer was indeed generated. In the syncytium-blocking assays (Table 1, experiments 2 and 3) this analogue was, in fact, more efficacious than DP-107, requiring one-half to one-fourth the concentration of the latter for inhibition. The increased activity exhibited by DP-125 has been reproduced in all assays performed to date and suggests that a dimer or higher-order multimer might actually represent the biologically active form of the peptide. In addition, CD measurements of DP-125 yielded ellipticity values similar to those of DP-107. Taken together, these observations indicate that the individual peptide components of the putative multimers are arranged in a parallel rather than antiparallel orientation.

Table 1. Test for peptide blockade of HIV-1_{LAI}-induced cell-cell fusion

Exp.	Peptide	No. of syncytia							
		Peptide, μ g/ml							
		50	25	12	6	3	1.5	0	
1	DP-31	85	80	78	87	90	75	89	
	DP-116	89	82	93	92	89	82	89	
	DP-107	0	0	0	0	46	80	89	
		40	20	10	5	2.5	1.25	0	
2	DP-107	0	0	0	36	83	98	93	
	DP-125	0	0	0	0	3	35	93	
3	DP-121	69	71	65	60	68	ND	76	
	DP-125	0	0	0	0	0	0	76	

To gain further insights into whether the solution structure observed for DP-107 in the CD and NMR studies is required for biological activity, the proline-containing analog (DP-121), which failed to exhibit helix-related CD signals at 37°C, was tested for activity in the cell fusion assay. The results showed no sign of inhibitory activity (Table 1). Although this finding does not prove that structure is necessary for biological activity, it is consistent with that possibility. In a similar fashion, each of the DP-107 peptide analogues tested that failed to block cell fusion also failed to show evidence of stable solution structure in CD studies. Also, GCN4-p1 (provided by R. Rutkowski, Whitehead Institute for Biomedical Research), a control peptide that is known to form a coiled coil structure similar to that of DP-107 but is unrelated in terms of primary sequence, exhibited no biologic activity.

Peptide Inhibition of Infection by Cell-Free Virus. The peptides were tested next for blockade of infection by cell-free virus. The results shown in Fig. 3 are representative of several experiments in which the DP-107, -125, and -116 (CS3) peptides were compared for potency in the blockade of HIV-1_{LAI} infection of AA5 cells. Each concentration of peptide was incubated in triplicate with virus (about 500 TCID₅₀) and cells. After 7 days of culture, cell-free supernatant was tested for RT activity. The results (Fig. 3) demonstrate that both DP-107 and DP-125 inhibited the *de novo* infection process at about the same effective doses as noted in the fusion assays described above. Moreover, the dose required for inhibitory effect was lower for the disulfide-bridged DP-125 analogue, and no antiviral effect was noted for DP-116.

Test For Direct Virocidal Effect. At this time, it is difficult to envision a mechanism by which the gp41 synthetic peptides inhibit infection and cell-cell fusion. As a first step, we asked whether the peptides acted directly on the virus particles. For this purpose, HIV-1_{LAI} was incubated for 90 min with DP-107 at 40 µg/ml. Parallel portions of the virus stock were incubated either with medium alone or with soluble CD4 at 10 µg/ml. Virus in each of these three treatment groups was then pelleted through a 5% sucrose cushion to remove excess inhibitor from the virus. Pelleted virus was then diluted in inhibitor-free medium and surviving virus was titrated on the AA5 cells for infectivity. DP-107, even at 40 µg/ml, had little or no effect on infectious titer (Fig. 4). In contrast, we observed inhibition when DP-107 was left in the culture with cells and virus, even at the high virus dose of 2.6×10^5 infectious units. These results suggest that a primary effect of the peptides on cell-free virions is unlikely. The results of pretreatment with DP-107 contrasted sharply with the dramatic, irreversible effect of soluble CD4 on the HIV-1_{LAI} virions, which may be related to shedding of gp120 from certain prototypic isolates after treatment with CD4 analogues (16, 17).

Peptide Inhibition of PBMC Infection by Primary Isolates. Substantial differences in sensitivity to antiviral agents can exist between laboratory-adapted prototypic isolates and primary field isolates passaged only through PBMCs. This

FIG. 3. Test for peptide blockade of AA5 cell infection by HIV-1_{LAI}. About 500 TCID₅₀ of HIV-1_{LAI} was added to 2×10^4 AA5 cells and test peptides (final concentrations shown) in a final volume of 100 µl. Cell cultures were maintained in 96-well microtiter plates for 8 days by addition of fresh medium (but no further addition of peptides) every other day. On the day 8, supernatant was tested for RT activity as evidence of successful infection.

FIG. 4. Test for direct virocidal effect of peptides and soluble CD4 (ST4). The HIV-1_{LAI} stock was divided into two portions. Samples of one portion (shown in figure as virus pelleted +) were treated for 2 hr at 37°C in medium alone, with DP-107 at 40 µg/ml, or with ST4 at 10 µg/ml. Virus was then pelleted through a 5% sucrose layer to separate virus from nonassociated inhibitor. The virus-containing pellets were dispersed in medium and serial dilutions were tested for infectivity on the AA5 cells. Serial 4-fold dilutions of the other portion of virus (virus pelleted -) were tested directly for infection of the cells, with each dilution of virus incubated in the absence or presence of DP-107 at 40 µg/ml.

problem was first highlighted by Ho and colleagues (18) in studies with soluble CD4. To test whether the gp41 peptides might exhibit a similar discordance in reactivity, the DP-107, DP-125 (cysteine analogue), and sT4 (same reagent as used in the preceding experiment) were compared for inhibition of PBMC infection by two primary HIV-1 isolates and HIV-1_{LAI}. The results (Fig. 5) show that the peptides inhibit both the primary and the prototypic isolates tested. Only a single dose of soluble CD4 (10 µg/ml) was included for comparative purposes, and it is apparent that this reagent was substantially more active on the cell line-adapted HIV-1_{LAI} than on the two primary isolates.

DISCUSSION

These studies provide direct support for models of the HIV TM protein that predict residues 558–595 of the envelope gp41 represent an amphipathic α -helical fiber and that hydrophobic interactions of these fibers to form coiled coils might play a role in envelope multimerization (2, 3). The synthetic peptide analogue of this region, DP-107, exhibited a high degree of stable helical structure as estimated by CD,

FIG. 5. Comparison of peptides and soluble CD4 to inhibit primary virus isolates. About 25 TCID₁₀₀ of two primary isolates and HIV-1_{LAI} were added to suspensions of phytohemagglutinin-activated human PBMCs (about 1.5×10^6 per ml) containing test peptide (40 or 10 µg/ml) or soluble CD4 (ST4, 10 µg/ml). Each treatment condition was tested in duplicate, and the cells were cultured in RPMI 1640 containing 10% (vol/vol) fetal bovine serum and 5% (vol/vol) interleukin 2 (supplied by Cellular Products, Inc.). Four days postinfection an equal number of fresh PBMCs were added to each well. This step allows for further expansion of virus and increases the noise-to-background ratio in the final RT assay. Supernatant was harvested 8 days after infection and tested for RT activity.

with a T_m of 72°C for a 10 μ M solution. This value compares with one of about 55°C at a similar concentration for the GCN4 peptide model described by O'Shea *et al.* (4). In both cases the T_m values were concentration-dependent as expected in systems where secondary structure is stabilized by self-association (4). Preliminary sedimentation equilibrium experiments also support this conclusion (W. Stafford and C.W., unpublished work). Methods which will allow accurate estimates of the equilibrium constants associated with peptide oligomerization need to be developed. This information is also necessary for NMR studies to establish the solution structure of the peptide.

In contrast to the stable solution structure observed for DP-107, which was consistent with the predictive models, the biological activity of the peptide was unexpected and its mechanism is not readily apparent. The results shown in Fig. 4 suggest that the peptide does not act directly on the cell-free virions. Qureshi *et al.* (13) have reported that an overlapping peptide, CS3 (DP-116 in this study as shown in Fig. 1), inhibited infection when coupled to albumin and suggested that this effect occurred by attachment to a second receptor on the cell surface required for membrane fusion. Those investigators tentatively identified a candidate for the receptor as a 44-kDa protein. Although such a mechanism would be consistent with the DP-107 result shown in Fig. 4, other observations argue that these two peptides are quite dissimilar and thus might function through different mechanisms. Most importantly, the CS3 peptide was active only after conjugation to albumin, which contrasts with the striking antiviral effect of the free, nonconjugated DP-107 peptide. Moreover, the CS3 peptide (DP-116 in Fig. 2) showed no evidence of stable secondary structure by CD even at low temperatures and high concentrations. Our experiments suggest that either structure or the capacity to assume stable secondary structure is a requirement for biological activity. For example, the DP-107 analogue containing a helix-breaking proline substitution (DP-121) and several truncated versions of DP-107 (data not shown) that either disrupted or failed to show stable solution structure did not exhibit antiviral activity. Nevertheless, we cannot rule out the possibility that the CS3 peptide assumes structure when conjugated to a carrier protein or that conjugation in some way removes the apparent structural requirement for activity.

Several other mechanistic possibilities have yet to be tested. One of these might involve disruption of the virus/cell membrane fusion processes after the initial gp120-CD4 binding step. According to this hypothesis, conformational changes induced by CD4 binding would allow access of DP-107 to its homologous sequence in gp41 and in some way disrupt gp41-mediated fusion. Such a mechanism would be consistent with the results shown in Fig. 4 and might be tested by using the CD4-resistant primary isolates shown in Fig. 5. We also have not ruled out the possibility that the peptides inhibit at a post-entry step in the virus life cycle. For example, it is conceivable that the interaction of peptide with envelope precursors might have a disruptive effect on maturation of the envelope and on virus morphogenesis.

The region of the HIV-1 TM envelope protein defined by DP-107 includes and/or overlaps with sites reported to exhibit a variety of biological activities. These include immunosuppression (19, 20), complement activation (21), escape from neutralizing antibody (22), and humoral immunodominance (14, 15, 22, 23, 24). Further development of DP-107 as a potential therapeutic agent will have to take these properties into account, particularly the immunosuppressive features. Nevertheless, DP-107 and appropriately modified analogues represent attractive candidates for further development. The potency with which they inhibit viral replication

and cell-cell fusion in the HIV-1 model (IC_{50} of $\approx 0.2 \mu$ M for the DP-125 analogue) compares favorably on a molar basis with that of 3'-azido-3'-deoxythymidine and other dideoxynucleosides (1). When studied in the same assays with recombinant CD4, these peptides exhibited the added feature of effectively inhibiting primary isolates. The highly conserved structural features of this region are not only important for HIV-1 but potentially applicable to a broad range of fusogenic viruses. Whether or not it will be possible to take advantage of these characteristics by direct use of the peptides as therapeutic agents will remain uncertain until studies of toxicity and pharmacokinetics are conducted. In the meantime elucidation of the *in vitro* antiviral mechanisms coupled with the structural information derived from these peptides should contribute valuable insights into the structure and function of the HIV-1 envelope.

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