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Peptides corresponding to a predictive α-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection

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ABSTRACT **To define the role of the human immunodeficiency virus type 1 (HIV-1) envelope proteins in virus infection, a series of peptides were synthesized based on various regions of the HIV-1 transmembrane protein gp41. One of these peptides, DP-178, corresponding to a region predictive of α-helical secondary structure (residues 643–678 of the HIV-1_{LAI} isolate), has been identified as a potent antiviral agent. This peptide consistently blocked 100% of virus-mediated cell–cell fusion at <5 ng/ml (IC_{90} ≈ 1.5 ng/ml) and gave an ≈10 times reduction in infectious titer of cell-free virus at ≈80 ng/ml. The inhibitory activity was observed at peptide concentrations ≈10^4 to 10^5 times lower than those at which cytotoxicity and cytostasis were detected. Peptide-mediated inhibition is HIV-1 specific in that ≈10^2 to 10^3 times more peptide was required for inhibition of a human immunodeficiency virus type 2 isolate. Further experiments showed that DP-178 exhibited antiviral activity against both prototypic and primary HIV-1 isolates. As shown by PCR analysis of newly synthesized proviral DNA, DP-178 blocks an early step in the virus life cycle prior to reverse transcription. Finally, we discuss possible mechanisms by which DP-178 may exert its inhibitory activity.**

The transmembrane (TM) protein (gp41) of human immunodeficiency virus type 1 (HIV-1) plays a key role in both virus-mediated cell–cell fusion and infection by cell-free virus. Reports have implicated various domains of gp41 with specific aspects of these processes (1–7). For example, the N terminus of gp41 (residues 517–532 of HIV-1_{LAI}) serves as a fusogenic domain whose function probably involves insertion into and disruption of the target cell membrane (1, 4). The membrane-spanning and cytoplasmic domains (residues 689–710 and 711–861, respectively) affect the fusion phenotype in HIV-1, human immunodeficiency virus type 2 (HIV-2), and simian immunodeficiency virus (5, 8–10). Residues 558–595 model a coiled-coil (leucine zipper-like) structure that may be critical for fusion-related conformational changes in gp41 (11–13). Also using synthetic peptides, investigators have described two sites within gp41 (residues 583–599 of HIV-1_{BH10} and residues 651–665 of HIV-1_{LAI}, respectively) that may interact with putative non-CD4 cellular receptors for HIV-1 (14, 15). In addition, a discontinuous region within gp41 (residues 534–544 and 644–650) has been identified as an element of gp120–gp41 association (16).

Synthetic peptides modeling specific domains within the HIV-1 envelope display various degrees of antiviral activity (17–19). Recently, Jiang *et al.* (20) reported that a gp41-derived synthetic peptide (termed peptide 637–666) inhibited virus-mediated cell–cell fusion and infection by cell-free virus at levels as low as 1 μg/ml. We have demonstrated (11) that two peptides, DP-107 and DP-125, modeling the leucine-zipper-like region of gp41, exhibited stable α-helical structure under physiological conditions and inhibited HIV-1 infection by both cell-free virus and cell–cell fusion. Also, viruses containing secondary-structure-disrupting mutations within this region were severely impaired in their ability to initiate infection (12, 13). The antiviral activity of certain of these peptides may be due to their ability to accurately model and interact with functional protein domains.

We have synthesized additional peptides corresponding to linear fragments of gp41 to identify other regions of the HIV-1 TM domain that may prove useful in understanding the role of this protein in virus infection. One of these peptides, DP-178, was modeled after a region of gp41 that was predicted to be rich in secondary structure (Fig. 1) (22). While this material failed to exhibit stable solution structure under physiologic conditions (C.T.W., unpublished data), it did prove to be a potent inhibitor of HIV-1-mediated cell–cell fusion. We have reported (23) that DP-178 completely inhibited HIV-1_{LAI}-mediated cell–cell fusion at concentrations as low as 1 ng/ml (≈200 pM). To our knowledge, it is rare for synthetic peptides to exhibit HIV-1-specific activity at such low concentrations.

In this report, we evaluate the ability of DP-178 to block infection by cell-free virus. We also assess the specificity of the peptide's inhibitory activity against several prototypic and primary HIV-1 isolates and a HIV-2 isolate. Using peptide analogs, we establish both the primary amino acid sequence and minimum length requirements for inhibition. Finally, we identify the point in HIV-1 replication that is disrupted by DP-178 and discuss possible mechanisms through which this material may exert its inhibitory activity.

MATERIALS AND METHODS

Peptide Synthesis. Peptides were synthesized, purified, and characterized as described (11). All peptides were acetylated at the N terminus and amidated at the C terminus. Lyophilized peptides were stored desiccated and peptide solutions were made in water or PBS at ≈1 mg/ml with concentrations determined from A_{280} (24).

Virus. HIV-1 isolates were obtained from R. Gallo (National Cancer Institute, Bethesda, MD), J. Levy (University of California at San Francisco), and J. Bradac (National Institute of Allergy and Infectious Diseases, Bethesda, MD). HIV-2_{NIHZ} was obtained from D. Zagury and R. Gallo (National Cancer Institute). High-titered virus stocks were prepared in CEM cells as described (11). Infectious titers were estimated by end-point dilution on AA5 and CEM cells.

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Abbreviations: HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; PBMC, peripheral blood mononuclear cell; TM, transmembrane; TCID, tissue culture infectious dose; RT, reverse transcriptase.

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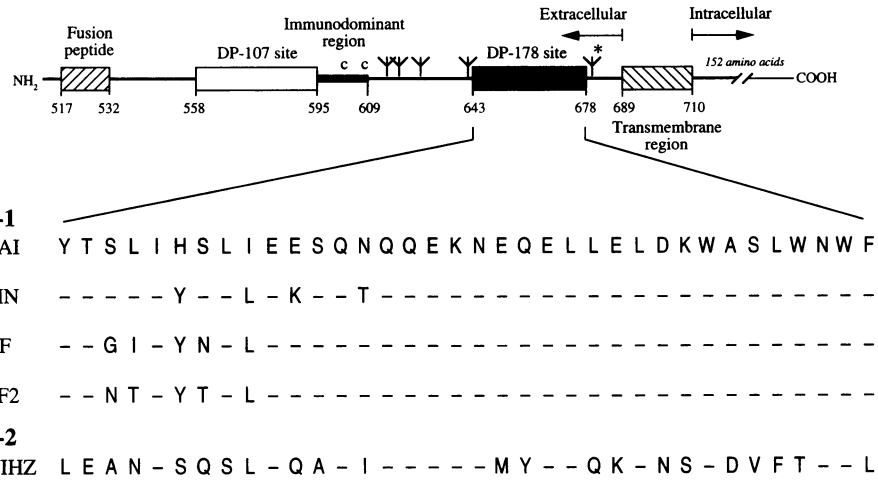

FIG. 1. Schematic representation of the HIV-1_{LAI} TM protein gp41 and the variation in the DP-178 region among several prototypic HIV-1 isolates and the analogous region from HIV-2_{NIHZ}. Amino acid residues are numbered according to ref. 21. The asterisk indicates that although this is a consensus glycosylation site, it has not been established that this site is glycosylated.

Reverse transcriptase (RT) activity present in the supernatants was taken as criteria for successful infection. The 50% tissue culture infection dose ($TCID_{50}$) was calculated by using the formula of Reed and Muench (25). Primary HIV-1 isolates 596 and 598 were expanded in activated peripheral blood mononuclear cells (PBMCs) from normal donors.

Infectivity Assay. The ability of the peptides to inhibit infection with prototypic cell-free virus was evaluated by incubating serial dilutions of cell-free virus with AA5 or CEM target cells containing various concentrations of peptide. Peptides were tested against the two primary isolates and the prototypic HIV-1_{LAI} isolate in a similar assay using PBMCs as target cells. Both assays were carried out as described (11).

Cell–Cell Fusion Inhibition Assay. The ability of the peptides to block virus-mediated cell–cell fusion was assessed (11).

PCR Analysis of Newly Synthesized Proviral DNA. Newly synthesized HIV-1 DNA was detected by a PCR-based assay (26). Briefly, 10^6 CEM cells were incubated with peptide, Leu3A, or PBS for 30 min at 37°C. Cells were then infected with HIV-1_{LAI} pretreated with DNase I. After a 2-hr incubation at 37°C, cells were extensively washed with PBS, trypsinized, washed three more times in PBS, and incubated in growth medium for 18 hr at 37°C. Cells were lysed overnight at 37°C in lysis buffer. Cellular lysates (10 μl each) were subjected to 32 cycles of PCR amplification using primers (each at 1.5 μM) corresponding to the envelope gene (nt 8838–8358) and the U3 region of the 3′ long terminal repeat (nt 9533–9558). PCR amplification was performed using *Taq* DNA polymerase (Promega). Each amplification cycle consisted of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and elongation for 1 min at 72°C.

RESULTS

Peptide Inhibition of Syncytium Formation Induced by Infected Cells. The ability to block syncytium formation between cells expressing HIV-1 envelope and uninfected CD4-expressing target cells (virus-mediated cell–cell fusion) has served as a useful screen for antiviral reagents that interfere with virus entry (i.e., neutralizing antibodies) (27). The DP-178 peptide proved to be an extremely potent inhibitor of this process when compared with other peptide inhibitors that also block HIV-1-mediated cell–cell fusion. As shown in the representative experiment depicted in Fig. 2, DP-178 gave an IC_{90} value of 1.4 ng/ml (300 pM, IC_{50} = 0.5 ng/ml or 100 pM) against the HIV-1_{LAI} isolate. The peptide gave a 90% reduction in syncytium formation at concentrations $\approx 10^2$ to 10^3 times lower than other tested peptide inhibitors based on gp41 envelope sequences, including the coiled-coil peptides DP-107 and DP-125 (11) and the peptide 637–666 (DP-219) described by Jiang *et al.* (20) that shares partial sequence homology with DP-178 (discussed below). Also, DP-180, a control peptide containing a scrambled DP-178 sequence, failed to demonstrate antiviral activity. These results also compare favorably with nonpeptide inhibitors of HIV-1-mediated fusion, as measured in the same assay including soluble forms of CD4 ($IC_{90} \approx 100$ nM; data not shown) and the anti-CD4 human monoclonal antibody Leu3A ($IC_{90} \approx 5$ nM; data not shown).

The DP-178 peptide, which is based on the HIV-1_{LAI} envelope sequence, exhibited similar levels of inhibition when evaluated against a panel of prototypic HIV-1 isolates (Table 1). These results establish that although peptide activity varied as a function of the HIV-1 isolate (e.g., IC_{90} for HIV-1_{LAI} = 6 ng/ml and IC_{90} for HIV-1_{MN} = 51 ng/ml), the degree of variability was relatively small in contrast to the isolate-specific activity exhibited by other viral inhibitors (e.g., soluble CD4 or neutralizing antibody) (28). Also, from the experiment shown in Table 1, we concluded that the inhibitory activity of DP-178 was HIV-1-specific, requiring $\approx 10^3$ to 10^4 times more peptide to obtain a similar level of inhibitory activity against the HIV-2_{NIHZ} isolate.

Peptide Determinants Required for Antiviral Activity. To map the peptide determinants necessary for activity, a series of truncation and extension analogs of DP-178 were prepared and compared with the parent compound for their ability to block virus-mediated cell–cell fusion. The results from these experiments are summarized in Fig. 3. Due to considerable

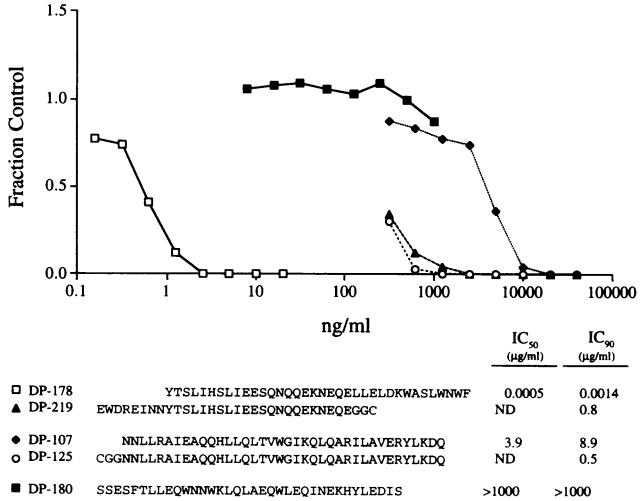

FIG. 2. Blockade of HIV-1_{LAI}-induced cell–cell fusion by several gp41-derived peptides. DP-219 is identical to peptide 637–666 described by Jaing *et al.* (20). ND, not done.

Table 1. DP-178 inhibition of cell–cell fusion—comparison of activity of HIV-1 isolates and HIV-2_{NIHZ}

Virus	Peptide, ng/ml IC_{50}	IC_{90}
HIV-1_{LAI}	2	6
HIV-1_{MN}	15	51
HIV-1_{RF}	7	24
HIV-1_{SF2}	2	5
HIV-2_{NIHZ}	3400	9600

sequence variability observed within the N terminus of the DP-178 region (Fig. 1), a series of peptides corresponding to N-terminal deletions were synthesized. Peptides containing 1- and 3-residue deletions from the N terminus, DP-211 and DP-210, respectively, gave IC_{90} values against HIV-1_{LAI} of ≈17 ng/ml. While this value is nearly 10 times higher than that required to give similar results to DP-178, these peptides are still potent inhibitors of cell–cell fusion. In contrast, deletion of 5 residues from the N terminus resulted in a dramatic decrease in antiviral activity. This truncated peptide, DP-209, gave an IC_{90} value of 400 ng/ml or ≈$10^{2.5}$ times higher than the parent compound.

Deletions at the highly conserved C terminus completely abolished peptide-mediated antiviral activity. A 4-residue C-terminal truncation resulted in a peptide, DP-213, that did not block fusion even at 1 μg/ml. This result suggests a major role for the highly conserved C-terminal region of DP-178 in the observed antiviral activity. Interestingly, an extension of 9 residues at the N terminus, DP-207, gave a peptide with inhibitory activity similar to the prototype. This series of experiments demonstrates that little divergence from the original DP-178 sequence can be tolerated if antiviral activity in the picomolar range is to be retained. However, truncations are more readily accommodated at the N terminus than at the C terminus, which is consistent with the relative degrees of sequence variation within these regions of the HIV-1 envelope (Fig. 1).

Peptide Inhibition of Infection by Cell-Free Virus. Several experiments were performed to assess the ability of DP-178 to block infection of CD4-expressing cells by cell-free virus. Fig. 4 shows the results from a representative experiment using the laboratory-adapted isolates HIV-1_{LAI} and HIV-2_{NIHZ}. As shown in Fig. 4, the peptide was much more active against the HIV-1 isolate than the HIV-2 isolate. Consistent with results from the cell–cell fusion assay, inhibition of infection by HIV-2_{NIHZ} required ≈10^2 times more peptide than was needed to achieve the same result with HIV-1_{LAI}. As expected, a control peptide DP-116 (11) exhibited no inhibitory activity. Although DP-178 proved to be an effective inhibitor of cell-free virus infection in the HIV-1 system (V_n/V_o = 0.1 at 80 ng/ml; where V_n is the virus titer in the presence of peptide and V_o is the virus titer in the absence of peptide), the concentration of peptide required to achieve this result (as measured by RT assay) was ≈10 to 10^2 times higher than the concentration required to give similar levels of inhibition in the cell–cell fusion assay (compare Tables 1 and 2).

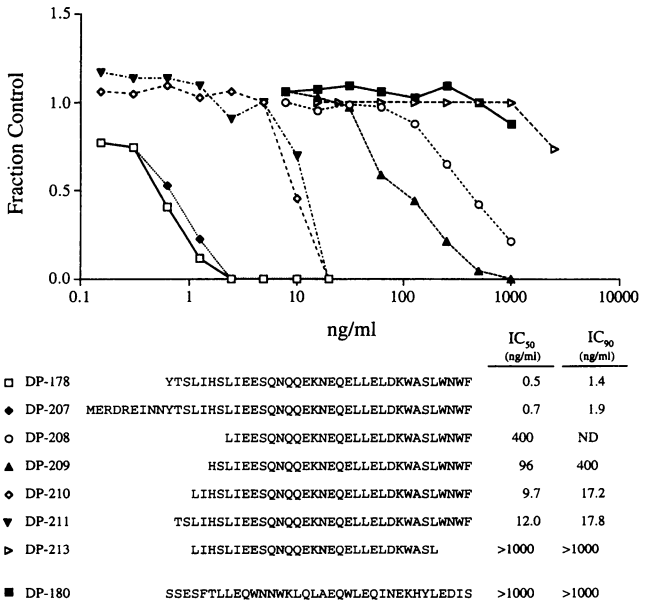

FIG. 3. Blockade of HIV-1_{LAI}-induced cell–cell fusion by DP-178 and analogs.

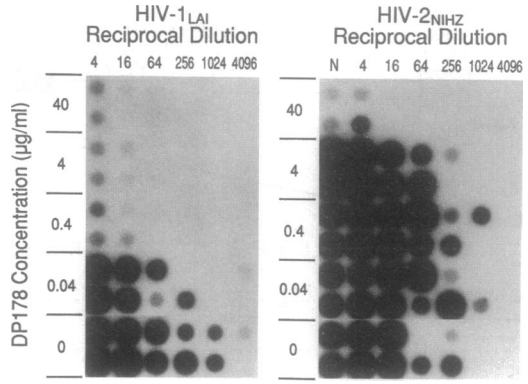

FIG. 4. Test for peptide blockade of CEM cell infection by HIV-1_{LAI} and HIV-2_{NIHZ}. Serial dilutions of the two virus stocks were treated with no peptide or with DP-178 at four concentrations in duplicate. The virus/peptide mixtures were allowed to incubate at room temperature in a 96-well microtiter plate for 1 hr, and then 2 × 10^5 CEM cells per well were added. The cultures were incubated for 8 days, with fresh medium added every other day. On day 8, supernatant was tested for RT activity as evidence of a successful infection. The radioactivity was estimated using a Packard Matrix counter and used for the calculations shown in Table 2.

Unlike certain inhibitors of HIV-1 infection (e.g., soluble forms of CD4) (28), the antiviral activity of DP-178 was not limited to prototypic viruses. As shown in Table 3, comparable levels of peptide inhibited both the laboratory-adapted HIV-1_{LAI} isolate and two primary HIV-1 isolates, HIV-1_{596} and HIV-1_{598}. The level of peptide required for inhibition appears to be greater in the PBMC-based assays than in assays carried out in continuous cell lines (e.g., AA5 or CEM) for both the prototypic and primary HIV-1 isolates (compare Table 2 and Table 3).

DP-178 Blocks a Step in Virus Replication That Occurs Prior to Provirus Formation. A PCR-based assay adapted from the

Table 2. DP-178 concentrations required to inhibit infection with cell-free virus

Virus	Peptide, μg/ml V_n/V_o = 0.1 DP-116	DP-178	ID_{50} DP-116	DP-178
HIV-1_{LAI}	>40	0.08	>40	0.09
HIV-2_{NIHZ}	>40	7.4	>40	26

Values were calculated from the experiment shown in Fig. 4 after estimation of RT cpm in each position of the filter with a β counter. The peptide concentrations required to reduce the infectious titer ($TCID_{50}$) by one order of magnitude (V_n/V_o = 0.1) is shown. For the ID_{50} value, the cpm in the duplicate wells shown in Fig. 4 were averaged and plotted against peptide concentration. The peptide concentration that gave a 50% reduction in RT cpm was interpolated as the ID_{50} value. The estimate of virus infectious titer at the virus dilution used (1:4) for this calculation was 512 $TCID_{50}$ for HIV-1_{LAI} and 32 $TCID_{50}$ for HIV-2_{NIHZ}.

Table 3. Test for inhibition of primary HIV-1 isolate infection of PBMCs

Peptide	ID_{50}, μg/ml		
	HIV-1_{596}	HIV-1_{598}	HIV-1_{LAI}
DP-178	2.8	1.2	1.1
DP-180	>20	>20	>20

Dose of peptide yielding a 50% reduction in RT cpm is shown. Serial dilutions of peptides were tested in replicates of five wells for each dilution. Input virus concentration was kept constant at ≈84 $TCID_{50}$ for HIV-1_{LAI}, 187 $TCID_{50}$ for HIV-1_{596}, and 260 $TCID_{50}$ for HIV-1_{598}.

procedure described by Zack *et al.* (26) was utilized to determine whether DP-178 inhibits virus infection at an early or late stage of HIV replication. Newly synthesized proviral DNA in virus-challenged CEM cells was assessed by PCR amplification 18 hr after infection, thus simulating a single round of virus replication. As shown in Fig. 5, the presence of DP-178 during initial HIV-1_{LAI} infection of CEM cells dramatically reduced the levels of newly synthesized proviral DNAs detectable by PCR amplification. The coiled-coil peptide DP-107 and the CD4-specific Leu3A monoclonal antibody provided similar reductions in HIV-1-specific DNA synthesis. Pretreatment with control peptides DP-180 or DP-121 (the Pro mutant analog of DP-107) (11) failed to reduce provirus formation. These results indicate that DP-178 interferes with a step in the virus life cycle that occurs prior to provirus formation, probably during virus entry and/or uncoating.

DISCUSSION

DP-178 exhibited remarkable potency in inhibiting both virus-mediated cell–cell fusion and infection by cell-free virus. The peptide gave a 90% reduction in syncytium formation and infection by cell-free HIV-1_{LAI} at ≈200 pM (1–2 ng/ml) and 18 nM (80 ng/ml), respectively. Similar results were obtained with other prototypic HIV-1 isolates (MN, RF, and SF2) and two primary isolates that had not been cultured in cell lines. The other peptide inhibitors evaluated in this study (DP-107, DP-125, and DP-219) required concentrations ≈10 to 10^3 times higher to achieve similar effects (Fig. 2). This antiviral activity is not due to cytotoxicity or cytostasis, as inhibitory concentrations were at least ≈10^4 to 10^5 times lower than those required to visualize toxic or cytostasis effects in tissue culture (data not shown). Also, peptides corresponding to analogous DP-178 sequences of the HIV-1 isolates MN, RF, and SF2 similarly blocked cell–cell fusion

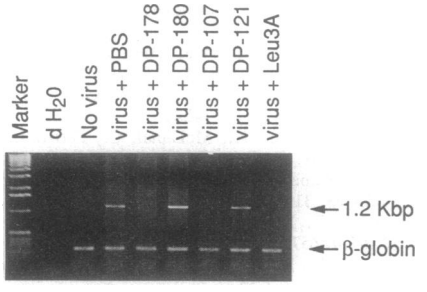

FIG. 5. PCR analysis of newly synthesized proviral DNA in CEM cells. Approximately 10^6 CEM cells were pretreated with synthetic peptides (20 μg/ml), the human monoclonal antibody Leu3A (10 μg/ml), or PBS and then infected with HIV-1_{LAI} (≈10^5 $TCID_{50}$) for 18 hr. Cellular DNAs were prepared and analyzed by PCR amplification using primers specific for HIV-1 and β-globin. PCR products were visualized after electrophoresis through a 1% agarose gel. dH_2O, distilled H_2O.

mediated by both homologous and heterologous virus (data not shown).

DP-178 was a more potent inhibitor of cell–cell fusion than of infection by cell-free virus (compare Tables 1 and 2). This observation was unexpected as in our experience the concentration of inhibitor required to block fusion of infected cells is usually equivalent to or, in the case of neutralizing antisera, greater than that required to block cell-free virus infection. Although, the reason for the reversal in potency of DP-178 in these two assays is unclear, it may reflect differences in the two modes of infection (e.g., cell-free virus transmission vs. cell–cell transmission).

The inhibitory activity afforded by DP-178 is specific for HIV-1. A $>10^2$ times difference in potency was observed against HIV-1 compared with HIV-2 in cell–cell fusion and infectivity assays (Tables 1 and 2). Also, DP-178 failed to inhibit syncytium formation in the related retrovirus system human T-cell leukemia virus type I (data not shown). The specificity of DP-178 for HIV-1 compared with HIV-2 could be related to differences in the envelope sequences of the corresponding regions of the two viruses. Comparison of the TM sequences of HIV-1_{LAI} and HIV-2_{NIHZ} (Fig. 1) revealed that these regions have limited sequence homology, sharing only 15 of 36 residues (a sequence homology of 42%). Also, the control peptides DP-180 (Figs. 2 and 3) and DP-116 (Table 2) showed no detectable antiviral activity. Although DP-178 corresponds to the gp41 sequence of the LAI isolate, its antiviral activity was not isolate-restricted, exhibiting similar levels of activity against the heterologous HIV-1 isolates RF, MN, and SF2 in the fusion assay (Table 1).

In the infectivity assays, differences in peptide activity as a function of the target cell (i.e., primary PBMCs vs. transformed T cells) were observed (compare Tables 2 and 3). In the PBMC-based assay, peptide concentrations of ≈ 1 μg/ml were required to achieve inhibitory levels similar to that of DP-178 at ≈80 ng/ml in the identical experiment with CEM cells. We conclude that this difference is due to the target cells rather than the virus or peptide since similar peptide concentrations were required to inhibit infection of PBMCs by the prototypic HIV-1_{LAI} and the two primary isolates (Table 3).

Other investigators have implicated the DP-178 region of gp41 in the fusion event. Using an envelope expression system, Cao *et al.* (29) assessed the effect of various nonconservative changes within this region of the TM domain on fusion phenotype. While many of the mutant envelopes were not fusion-competent, fusion in several mutants containing changes in the DP-178 domain was significantly enhanced over that observed with the wild-type envelope.

DP-178 contains the sequence ELDKWA (residues 662–667), which has been identified as the target epitope for the neutralizing human monoclonal antibody 2F5 (30). This finding suggests that the epitope lies on an exposed surface of the TM domain or that at some point the envelope undergoes a conformational change that renders this region accessible to neutralizing antibodies. Other reports suggest that residues contained within the DP-178 region can influence sensitivity to polyclonal neutralizing antisera (31) (M. Robert-Guroff, personal communication).

Various experiments were conducted to determine the mechanism through which DP-178 blocks virus infection. An assay to detect newly synthesized proviral DNA was used to determine whether an early or late stage of the virus life cycle is inhibited by DP-178. Under conditions favoring a single round of replication, DP-178 significantly inhibited HIV-1 provirus formation compared with the control peptide DP-180 (Fig. 5). This result suggests that DP-178 exerts its antiviral activity by interfering with virus entry or uncoating (early steps that occur prior to provirus formation) rather

than a late step such as envelope synthesis, processing, or assembly.

Additional ELISA-based peptide binding assays and flow cytometric analyses were used to determine whether DP-178 exerted its antiviral activity at the cellular or virus level. First, we evaluated the ability of DP-178 to disrupt the initial step of virus entry, the interaction of gp120 with its high-affinity receptor CD4 (32). Results from both assays confirmed that DP-178 did not disrupt this critical interaction (data not shown). We also investigated the possible interaction of the negatively charged DP-178 with the positively charged neutralization-sensitive V3 domain of gp120. In both the ELISA-based binding and flow cytometry assays, direct binding of DP-178 to the V3 region of gp120 was not detected, and peptide did not interfere with the envelope binding of the V3-specific monoclonal antibody 0.5β (data not shown).

Other experiments conducted in our laboratory suggested that DP-178 may inhibit virus entry by interacting with a distal region of the gp41 ectodomain. We have isolated a recombinant human Fab fragment that recognizes a discontinuous gp41 epitope containing both the DP-178 and DP-107 regions (C.-H. Chen, personal communication). CD studies showed that the stable α-helical structure of the DP-107 peptide was disrupted in a dose-dependent manner by addition of DP-178 (C.T.W., unpublished data), indicating a potential interaction between these two regions of gp41. We (11) and others (12, 13) have shown that this leucine-zipper-like domain is critical to virus entry, and perturbation of its structure can inhibit this process. Thus, these results suggest that peptides derived from the C terminus of the gp41 ectodomain (e.g., DP-178 region) may interfere with the infection process by interacting with sequences located near or within the leucine-zipper-like domain of the same molecule (e.g., DP-107 region).

Jiang *et al.* (20) have described an overlapping synthetic peptide referred to as peptide 637–666 that was also derived from the HIV-1 gp41 ectodomain and exhibited significant antiviral activity. The amino acid sequence of peptide 637–666 contains the N-terminal 22 residues of DP-178 and includes an additional 8 residues that are not found in DP-178 (Fig. 2). We have synthesized this peptide (DP-219) and have confirmed the original observations (Fig. 2). Jiang *et al.* (20) concluded that the antiviral activity of peptide 637–666 resulted from its interaction with the N-terminal fusion domain (FLGFL sequence) of gp41, which disrupts insertion of the fusogenic sequence into the target cell membrane during virus entry. Importantly, these studies and studies conducted by our group indicate that the target sites of DP-178 and peptide 637–666 are not identical. In support of this conclusion, two N-terminal residues that were found to be critical for peptide 637–666 antiviral activity are not present in DP-178 (Fig. 2). Also, extension of DP-178 to include the N-terminal 8 residues of the peptide 637–666 did not affect its activity (Fig. 3). Similarly, deletion of the C-terminal 4 residues of DP-178 (compare DP-210 and DP-213 in Fig. 3) resulted in a biologically inactive peptide, yet these residues are not present in peptide 637–666. Although potential interactions between DP-178 and the fusion domain of gp41 were not directly evaluated, these findings suggest that the determinants for biological activity for the two peptides are different.

The results presented here are consistent with the initial observations that DP-178 is a potent specific inhibitor of HIV-1-mediated cell–cell fusion (23) and infection by cell-free virus. However, perhaps more important than these observations is the prospect of using DP-178 as a probe for sites on the virus envelope or the host cell membrane that may serve as targets for antiviral development. Since virus infection can be completely inhibited at peptide concentrations in the picomolar to nanomolar range, the target site for DP-178 would seem to be both accessible and extremely sensitive to reagents that disrupt its role in the infection process. Therefore, it will be important to further define the mechanism involved in DP-178-mediated inhibition.

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1. Bosch, M. L., Earl, P. L., Fargnoli, K., Picciafuoco, S., Giombini, F., Wong-Stall, F. & Franchini, G. (1989) *Science* **244**, 694–697.
2. Dalgleish, A. G., Chanh, T. C., Kennedy, R. C., Kanda, P., Clapham, P. R. & Weiss, R. A. (1989) *Virology* **165**, 209–215.
3. Gabuzda, D., Olshevsky, U., Bertani, P., Haseltine, W. & Sodroski, J. (1991) *J. AIDS* **4**, 34–40.
4. Gallaher, W. R. (1987) *Cell* **50**, 327–328.
5. Helseth, E., Olshevsky, U., Gabuzda, D., Ardman, B., Haseltine, W. & Sodroski, J. (1990) *J. Virol.* **64**, 6314–6318.
6. Kowalski, M. L., Bergeron, L., Dorfman, T., Haseltine, W. & Sodroski, J. (1991) *J. Virol.* **65**, 218–291.
7. Bergeron, L., Sullivan, N. & Sodroski, J. (1992) *J. Virol.* **66**, 2389–2397.
8. Owens, R., Burke, C. & Rose, J. K. (1994) *J. Virol.* **68**, 570–574.
9. Gabuzda, D., Lever, A., Terwilliger, E. & Sodroski, J. (1992) *J. Virol.* **66**, 3306–3315.
10. Johnston, P. B., Dubay, J. W. & Hunter, E. (1993) *J. Virol.* **67**, 3077–3086.
11. Wild, C., Oas, T., McDanal, C., Bolognesi, D. & Matthews, T. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10537–10541.
12. Dubay, J. W., Roberts, S. J., Brody, B. & Hunter, E. (1992) *J. Virol.* **66**, 4748–4756.
13. Chen, S.-L., Lee, C.-N., Lee, W.-R., McIntosh, K. & Lee, T.-H. (1993) *J. Virol.* **67**, 3615–3619.
14. Henderson, L. A. & Qureshi, M. N. (1993) *J. Biol. Chem.* **268**, 15291–15297.
15. Chen, Y.-H., Ebenbichler, C., Vornhagen, R., Schulz, T. F., Steindl, F., Böch, G., Katinger, H. & Dierich, M. P. (1992) *AIDS* **6**, 533–539.
16. 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.
17. Owens, R. J., Tanner, C. C., Mulligan, M. J., Srinivas, R. V. & Compans, R. W. (1990) *AIDS Res. Hum. Retroviruses* **6**, 1289–1296.
18. Qureshi, M. N., Coy, D. H., Garry, R. F. & Henderson, L. A. (1990) *AIDS* **4**, 553–558.
19. Nehete, P. N., Arlinghaus, R. B. & Sastry, K. J. (1993) *J. Virol.* **67**, 6841–6846.
20. Jiang, S., Lin, K., Strick, N. & Neurath, A. R. (1993) *Biochem. Biophys. Res. Commun.* **195**, 533–538.
21. Myers, G., Korber, B., Berzofsky, J. A., Smith, R. F. & Pavlakis, G. N. (1991) *Human Retroviruses and AIDS 1992* (Los Alamos Natl. Lab., Los Alamos, NM).
22. Gallaher, W. R., Ball, J. M., Garry, R. F., Griffin, M. C. & Montelaro, R. C. (1989) *AIDS Res. Hum. Retroviruses* **5**, 431–440.
23. Wild, C., Greenwell, T. & Matthews, T. (1993) *AIDS Res. Hum. Retroviruses* **9**, 1051–1053.
24. Edelhoch, H. (1967) *Biochemistry* **6**, 1948–1954.
25. Reed, L. J. & Muench, H. (1938) *Am. J. Hyg.* **27**, 493–497.
26. Zack, J. A., Arrigo, S. J., Weitsman, S. R., Go, A. S., Haislip, A. & Chen, I. S.-Y. (1990) *Cell* **61**, 213–222.
27. Rusche, J. R., Javaherian, K., McDanal, C., Petro, J., Lynn, D. L., Grimaila, R., Langlois, A., Gallo, R. C., Arthur, L. O., Fischinger, P. J., Bolonesi, D. P., Putney, S. D. & Matthews, T. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3198–3202.
28. Daar, E. S., Li, X. L., Moudgu, T. & Ho, D. D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6574–6578.
29. Cao, J., Bergeron, L., Helseth, E., Thali, M., Repke, H. & Sodroski, J. (1993) *J. Virol.* **67**, 2747–2755.
30. Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Ruker, F. & Katinger, H. (1993) *J. Virol.* **67**, 6642–6647.
31. Back, N. K. T., Smit, L., Schutten, M., Nara, P. L., Tersmette, M. & Goudsmit, J. (1993) *J. Virol.* **67**, 6897–6902.
32. Dalgleish, A. G., Beverly, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) *Nature (London)* **312**, 763–767.