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Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion

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ABSTRACT **The synthetic peptides DP-107 and DP-178 (T-20), derived from separate domains within the human immunodeficiency virus type 1 (HIV-1) transmembrane (TM) protein, gp41, are stable and potent inhibitors of HIV-1 infection and fusion. Using a computer searching strategy (computerized antiviral searching technology, C.A.S.T.) based on the predicted secondary structure of DP-107 and DP-178 (T-20), we have identified conserved heptad repeat domains analogous to the DP-107 and DP-178 regions of HIV-1 gp41 within the glycoproteins of other fusogenic viruses. Here we report on antiviral peptides derived from three representative paramyxoviruses, respiratory syncytial virus (RSV), human parainfluenza virus type 3 (HPIV-3), and measles virus (MV). We screened crude preparations of synthetic 35-residue peptides, scanning the DP-178-like domains, in antiviral assays. Peptide preparations demonstrating antiviral activity were purified and tested for their ability to block syncytium formation. Representative DP-178-like peptides from each paramyxovirus blocked homologous virus-mediated syncytium formation and exhibited EC_{50} values in the range 0.015–0.250 μM. Moreover, these peptides were highly selective for the virus of origin. Identification of biologically active peptides derived from domains within paramyxovirus F_1 proteins analogous to the DP-178 domain of HIV-1 gp41 is compelling evidence for equivalent structural and functional features between retroviral and paramyxoviral fusion proteins. These antiviral peptides provide a novel approach to the development of targeted therapies for paramyxovirus infections.**

The Paramyxoviridae family encompasses three genera: the Paramyxovirus group, including parainfluenza types 1–4, mumps, Newcastle disease, Sendai, and simian type 5 viruses; the Morbillivirus group, including measles, canine distemper, and rinderpest viruses; and the Pneumovirus group, including human and bovine respiratory syncytial viruses (RSVs) and mouse pneumonia virus (1, 2). Many of these viruses are responsible for human disease. All members of the family contain two major surface glycoproteins, a receptor-binding protein (HN, H, or G), which facilitates attachment to cells, and a fusion (F) protein, which enables penetration of the virus genome into the host cell. Fusion of the paramyxovirus envelope with the cell membrane occurs at neutral pH and development of extensive syncytia in tissue culture is the characteristic cytopathologic effect (CPE) observed for many members of this virus family.

Fusion of the viral envelope or infected cell membranes with uninfected cell membranes is an essential step in the virus life cycle (3–5). The F glycoprotein mediates this function in paramyxoviruses, whereas in retroviruses fusion is mediated by the transmembrane (TM) protein. Both types of fusogenic proteins require activation by a proteolytic cleavage event mediated by a host cell enzyme (6, 7). Certain conserved features are common among the fusion proteins of different fusogenic viruses (3). First, since these are class I integral membrane proteins, a hydrophobic sequence near the C terminus anchors the protein in the virus envelope; second, a hydrophobic stretch of amino acids at the N terminus serves as the fusion domain and anchors the protein in the host cell membrane during the fusion process (3, 5). Interestingly, the fusion domains at the amino termini of RSV F_1 and human immunodeficiency virus type 1 (HIV-1) TM show a high degree of sequence homology (8).

Two intervening amphipathic heptad repeat regions have been identified in the fusion proteins of orthomyxoviruses, paramyxoviruses, and retroviruses (9, 10). Recently, mutational analyses and binding experiments have provided evidence for the interaction of these peptide regions in HIV-1 (11–13). These general domains within retrovirus TM proteins, paramyxovirus F proteins, coronavirus peplomer proteins, and influenza virus HA2 proteins have been reported to contain primary amino acid sequences predicted to form α-helices or coiled coils (5, 9–11, 14–16). The report of Gallaher *et al.* (14) first predicted the helical domains of the HIV TM protein (gp41). This work led to the discoveries of Wild *et al.* (11, 15, 17), who demonstrated that synthetic peptides derived from these potentially helical regions within the TM protein of HIV-1 were potent inhibitors of viral fusion and infection. One such peptide, DP-107, forms a helical coiled coil in aqueous solution at neutral pH. A second peptide, DP-178, demonstrates little or no helical structure in solution but exhibits potent and specific inhibition of HIV-1 fusion and infection (11). Similar results have been independently reported for an overlapping peptide (18, 19). We used a motif searching strategy, based on the known sequences of gp41 helical domains, that accurately predicted similar regions within the fusion proteins of a wide variety of enveloped viruses, including the paramyxovirus fusion proteins reported here.

Because of the functional and structural similarities between the HIV-1 TM protein and the F_1 subunit of the paramyxovirus fusion glycoproteins, we investigated the possibility that functional homologues of DP-107 or DP-178-like peptides could be derived from similar domains within the F_1 glycoproteins. Identification of similarly active peptides in the F proteins would provide additional evidence for conserved structural and functional features between the fusion proteins of retroviruses and paramyxoviruses.

We report here the identification of functional domains within the F_1 proteins of RSV, human parainfluenza virus 3 (HPIV-3), and measles virus (MV) that correspond to the DP-107 and DP-178 domains of HIV-1. We also describe peptides derived from paramyxovirus F_1 regions comparable to DP-178 that possess extremely potent and selective antiviral

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Abbreviations: CPE, cytopathic effect; DMSO, dimethyl sulfoxide; HIV, human immunodeficiency virus; HPIV, human parainfluenza virus; MV, measles virus; RSV, respiratory syncytial virus.

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activity *in vitro* against HPIV-3, RSV, and MV. Identification of paramyxovirus inhibitory peptides expands the initial observations of Wild *et al.* (15, 17) and demonstrates that antiviral peptides derived from discrete fusion protein domains can be found in other enveloped viruses. In addition, the potential of these peptides to be antiviral agents for medically important paramyxoviruses is discussed.

MATERIALS AND METHODS

Cells and Virus. Human RSV (Long strain), human MV (Edmonston strain), and HPIV-3 (strain NIH 47885) were obtained from the American Type Culture Collection (ATCC). The cell lines HEp2, Vero, and CV-1 were also obtained from ATCC. HEp2 cell monolayers persistently infected with HPIV-3 were made as described (20). Stocks of HIV-1_{LAI}, CEM, and MOLT-4 cells were kindly provided by T. Matthews (Duke University Medical Center).

Computerized Antiviral Searching Technology (C.A.S.T.). The primary amino acid sequences of the F proteins of RSV, HPIV-3, and MV were analyzed with a computer search strategy designed with the aid of a commercially available software package, PC/GENE. Starting with the amino acid sequences of HIV-1 gp41 peptides, DP-107 and DP-178, motifs were designed to search for amino acid sequences with similar characteristics within viral surface proteins, including paramyxovirus fusion proteins. Available viral protein sequences found in the Swiss-Prot database were scanned for matching motifs with the PC/GENE program PESEARCH.

Cytotoxicity and Viral CPE Assays. Crude peptides were tested for their ability to protect cell monolayers from viral CPE and to evaluate cytotoxicity in uninfected cells. The CPE and cytotoxicity assays were developed for RSV, HPIV-3, and MV and are based on cellular metabolism of a tetrazolium salt (XTT) to a water-soluble formazan dye (21).

Cell Fusion Assays. Virus-induced cell–cell fusion assays were used to assess the ability of peptides to block fusion. Cells which were acutely infected with either RSV, HPIV-3, or MV (multiplicity of infection, 3–5) were added to uninfected cell monolayers in the presence of peptides. Infected cells were dispersed at 24 hr postinfection by Versene (EDTA) treatment and washed, and a predetermined number of cells were added to uninfected cell monolayers in 96-well plates to give reproducible numbers of syncytia (generally 50–75 per well) in untreated control wells. Dilutions of peptides in complete medium were added to uninfected cell monolayers in 96-well plates, then infected cells were added to each well. After 18–24 hr of incubation at 37°C in a 5% CO_2 atmosphere, monolayers were fixed and stained with 0.5% (wt/vol) crystal violet in 100% methanol. Stained syncytial plaques were counted with a stereo dissecting microscope. A reduction in formation of syncytia (infectious centers) in comparison with an infected cell control containing no peptide is indicative of antiviral activity. A variation of this assay which used cell-free virions (50–100 plaque-forming units) in place of acutely infected cells gave similar results. Either procedure allows for quantitative measurement of cell-to-cell spread of infection mediated by the F protein of the respective paramyxovirus. Dose–response curves were generated and the 50% effective concentration (EC_{50}) for each peptide was determined. Fusion assays with HIV-1_{LAI} were performed as described (22).

Peptide Synthesis. Peptides were synthesized on a Rainin Symphony Multiplex multiple peptide synthesizer. Standard solid-phase synthesis techniques using fluorenylmethoxycarbonyl-protected amino acids were used (23, 24). All peptides were acetylated at the N terminus and amidated at the C terminus to enhance the biological half-life of the peptides (25). Cleavage of peptides from the resin and removal of side-chain blocking groups were automatically performed on the instrument with trifluoroacetic acid and the appropriate scavengers (5% thioanisol, 5% water, 2.5% ethanedithiol, 0.8 M phenol) (26). After cleavage, the peptide was precipitated with 4 volumes cold ether for 20 min, collected by centrifugation, washed twice with cold ether, and dried under vacuum for 24 hr. Lyophilized peptides were stored desiccated and peptide solutions were made in water or phosphate-buffered saline (PBS) or, if necessary, dimethyl sulfoxide (DMSO).

Peptide Purification and Characterization. Peptides were purified by reverse-phase HPLC on a Waters DeltaPak C_{18} column (25 mm × 300 mm, 15-μm particles) using a water and acetonitrile gradient containing 0.1% trifluoroacetic acid. All peptides were >95% pure by analytical reverse-phase HPLC. Amino acid analysis and electrospray mass spectroscopy confirmed the composition of the peptides.

Circular Dichroism. CD measurements were obtained with an Aviv Associates model 62DS spectropolarimeter calibrated with a standard solution of 10-camphorsulfonic acid (27). Samples contained 10–50 μM peptide in 0.1 M NaCl/10 mM potassium phosphate, adjusted to pH 7.0. For samples containing trifluoroethanol, the aqueous buffer was diluted 1:1 (vol/vol) with trifluoroethanol. Spectra were collected at 1°C with a 1.5-nm bandwidth, a 0.5-nm step size, and a 2.0-sec time constant in either 1- or 10-mm-path-length cells. Blank corrected data were smoothed with a third-order polynomial function. Sample temperature was regulated by a thermoelectric cell holder accurate to within 1°C.

RESULTS

A four-stage process was employed to identify biologically active peptides from the F proteins of paramyxoviruses. First, we used C.A.S.T. to analyze the primary amino acid sequences of the F proteins for domains that contained sequences similar in potential secondary structure characteristics to the DP-107 and DP-178 peptides of HIV-1 gp41. Second, we synthesized overlapping 35-residue peptides from the identified DP-178-like domains and tested the crude preparations for antiviral activity. Third, the peptides from active crude preparations were purified and retested for antiviral activity in virus fusion assays. Fourth, peptides were analyzed for helical structure by CD spectroscopy.

Computer Searches to Identify Heptad Repeat (HR) Domains. Based on the previously identified amino acid characteristics of peptides DP-107 and DP-178 from HIV-1 gp41 (15, 17), motifs were designed to search for amino acid sequences which are predicted to form helical secondary structure in other fusogenic viral proteins. The computer motif search selectively identified domains within gp41 that overlaid the amino acid sequences of DP-107 and DP-178 in all available HIV-1 sequences.

This approach allowed identification of biologically active peptides within both the HR1 and HR2 domains of the F_1 proteins from RSV, HPIV-3, and MV (Fig. 1). The following DP-107-like (HR1) and DP-178-like (HR2) regions were identified: RSV F_1, aa 149–206 (HR1) and aa 474–523 (HR2); HPIV-3 F_1, aa 119–183 (HR1) and aa 438–493 (HR2); MV, aa 116–191 (HR1) and aa 438–488 (HR2).

The identified sequences within HR1 and HR2 domains shared several characteristics with DP-107 and DP-178. The relative positions of these two domains—located next to the fusion peptide domain and the transmembrane anchor, respectively—were similar to the comparable domains in gp41 (Fig. 1). Within the RSV, HPIV-3, and MV F_1 glycoproteins, the search motif identified HR1 domains corresponding in position to the DP-107 coiled-coil domain of HIV-1, immediately downstream from the predicted fusion peptide sequences at their respective N termini. Similarly, regions corresponding to the DP-178 domain of HIV-1 TM were identified proximal to the predicted transmembrane region of each F_1 glycoprotein. The motif search also identified a unique

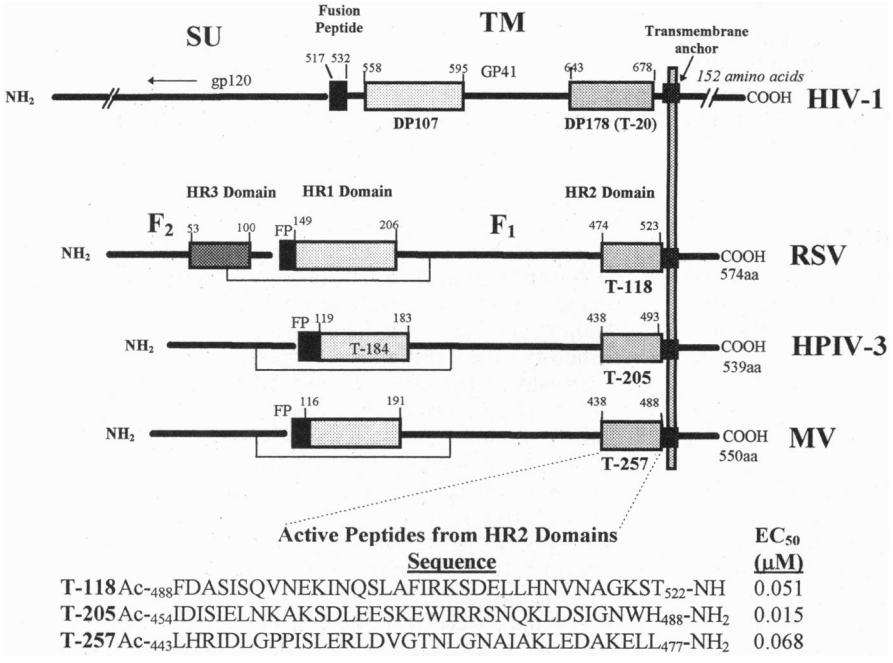

FIG. 1. Linear protein maps of HIV-1 TM compared with RSV, HPIV-3, and MV fusion (F) proteins. Designated DP-107-like (HR1) and DP-178-like (HR2) domains in the paramyxovirus F proteins are designated by amino acid number. An additional F_2 domain, designated HR3, was identified in RSV but not in HPIV-3 or MV. The predicted fusion peptide (FP) domains located at the N-terminal ends of the F_1 proteins are designated by their amino acid numbers and overlap with the N-terminal ends of predicted HR1 domains. The HR2 peptide sequences chosen for purification and further study for each virus are shown at the bottom. Peptides from each virus were tested in either antifusion assays or plaque reduction assays to determine the EC_{50} values (μM).

region within the RSV F_2 protein designated HR3 (aa 53–100; Fig. 1). No comparable HR3 domains were identified in either HPIV-3 or MV F_2 glycoproteins.

Peptide Scanning Across HR2 Domains to Locate Active Peptides. The two identified F_1 heptad repeat domains range in length from 47 to 75 aa and represent general regions within the F_1 proteins (Fig. 1). We chose a peptide scanning approach which required synthesis of overlapping peptides covering the HR1 and HR2 regions. Thirty-five amino acids (i.e., five heptad repeats) was chosen as a standard length for the peptides because the DP-107 and DP-178 peptides were of similar length (38 and 36 aa, respectively) and because this length was more efficiently synthesized than single peptides covering the entire region (i.e., 47–75 aa). Peptide scans across the HR1 domains were assayed and several demonstrated moderate antiviral activity (data not shown). Overlapping crude 35-aa peptides scanning across the HR2 domains showed significantly more potent antiviral activity when analyzed for their ability to protect cultures from viral CPE (Fig. 2). Note that peptides starting from the N-terminal area of each viral HR2 domain showed little or no activity (>100 μg/ml), whereas potent but variable antiviral activity was seen with the remaining peptides. We had previously determined that crude preparations of DP-107 and DP-178 were only 3–5 times less active than pure peptides and that these preparations were not cytotoxic at concentrations of up to 100 μg/ml, indicating that initial screening for active peptides would not require purification (data not shown). More than 95% of the crude preparations were soluble (1 mg/ml) in phosphate-buffered saline at pH 7. Less soluble peptides were dissolved in DMSO prior to analysis. No differences in antiviral activity were observed when DMSO was used to dissolve peptides which were also soluble in PBS (data not shown).

Antiviral Activity of Peptides Derived from HR2 Domains of F Proteins. Crude peptide preparations from HR2 were analyzed for their ability to prevent CPE in infectivity assays with RSV, HPIV-3, and MV. As shown in Fig. 2, peptides exhibited a wide range of antiviral activity in these assays. Because the crude preparations were synthesized and handled simultaneously, the inactive peptides served as negative controls. Several peptides from all three viruses demonstrated significant antiviral activity. Active peptide preparations from each virus were chosen for further study: T-118 from RSV, T-205 from HPIV-3, and T-257 from MV.

Active peptides were resynthesized, purified, and tested in infectious-center assays for their ability to block viral fusion. Fig. 3 shows the antiviral titration curves and cytotoxicity profiles of a representative active peptide for each virus: T-118 (RSV), T-205 (HPIV-3), and T-257 (MV). EC_{50} values (μM) were calculated for each peptide from triplicate assays. A representative dose–response curve is shown for each peptide and indicates that the antiviral activity seen is peptide dependent. Results of repeated assays showed that for RSV, peptide T-118 was the most active, with EC_{50} values of 0.050 μM. Several other peptides from this region had antiviral activity within the range 0.05–0.15 μM. The HPIV-3 peptide, T-205, inhibited fusion and infection at EC_{50} values as low as 0.015 μM. Against MV, T-257 exhibited antifusion activity with EC_{50} values as low as 0.042 μM.

Selectivity and Specificity of Antiviral Peptides. Three representative active peptides from the HR2 domains of these viruses were highly selective for their viruses of origin (Table 1). The EC_{50} values for selected peptides against their homologous virus were compared with values obtained when the peptides were tested against heterologous viruses. As shown in Table 1, no cross-inhibitory activity of any of the paramyxovirus DP-178-like peptides (up to about 25 μM) was detected for HIV-1. Likewise, neither DP-107 nor DP-178 was active against the paramyxoviruses. Peptide T-118 (RSV) and peptide T-257 (MV) were not active against HPIV-3, nor were their peptides active against each other at concentrations up to 100 μg/ml. However, the HPIV-3 peptide, T-205, was active against RSV at 1.76 μM, an ≈46-fold higher concentration than its activity against HPIV-3 (0.038 μM). T-205 blocked MV fusion at 1.19 μM, an ≈31-fold higher concentration than against HPIV-3. None of the peptides tested exhibited sufficient cytotoxic effects to determine 50% cytotoxic concentration (CC_{50}) values (i.e., >500 μg/ml). Thus, these peptides exhibit selectivity index values (CC_{50}/EC_{50}) of >1900 to >3000 against their homologous viruses.

Structural Analysis of HR1 and HR2 Peptides. Because the peptides described above were derived from F_1 domains predicted to have helical characteristics similar to DP-107 and DP-178, we analyzed representative peptides from each domain by CD for helical content. Purified peptide samples from HR1 and HR2 peptides were analyzed in PBS and the CD spectra for purified HPIV-3 peptides are shown in

RSV DP-178-LIKE REGION (F1)

		EC50 (ug/ml)
HR2:	G E P I I N F Y D P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N A G K S T T	
T-104	I I N F Y D P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K	91
T-105	I N F Y D P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S	93
T-106	N F Y D P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D	>100
T-107	F Y D P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E	20
T-108	Y D P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L	6
T-109	D P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L	8
T-110	P L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H	30
T-111	L V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N	9
T-112	V F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V	19
T-113	F P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N	8
T-114	P S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N A	6
T-115	S D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N A G	6
T-116	D E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N A G K	12
T-117	E F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N A G K S	13
T-118	F D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N A G K S T	6
T-119	D A S I S Q V N E K I N Q S L A F I R K S D E L L H N V N A G K S T T	8

HPIV3 DP178-LIKE REGION (F1)

		EC50 (ug/ml)
HR2:	Y T P N D I T L N N S V A L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W H Q S S T T	
T-189	Y T P N D I T L N N S V A L D P I D I S I E L N K A K S D L E E S K E	>100
T-190	T P N D I T L N N S V A L D P I D I S I E L N K A K S D L E E S K E W	>100
T-191	P N D I T L N N S V A L D P I D I S I E L N K A K S D L E E S K E W I	>100
T-192	N D I T L N N S V A L D P I D I S I E L N K A K S D L E E S K E W I R	>100
T-193	D I T L N N S V A L D P I D I S I E L N K A K S D L E E S K E W I R R	>100
T-194	I T L N N S V A L D P I D I S I E L N K A K S D L E E S K E W I R R S	62
T-195	T L N N S V A L D P I D I S I E L N K A K S D L E E S K E W I R R S N	72
T-196	L N N S V A L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q	1
T-197	N N S V A L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K	6
T-198	N S V A L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L	0.2
T-199	S V A L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D	2
T-200	V A L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S	1
T-201	A L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I	0.1
T-202	L D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G	0.03
T-203	D P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N	0.2
T-204	P I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W	0.07
T-205	I D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W H	0.11
T-206	D I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W H Q	2
T-207	I S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W H Q S	2
T-208	S I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W H Q S S	1
T-209	I E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W H Q S S T	2
T-210	E L N K A K S D L E E S K E W I R R S N Q K L D S I G N W H Q S S T T	2.4

MV DP178-LIKE REGION (F1)

		EC50 (ug/ml)
HR2:	P D A V Y L H R I D L G P P I S L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q I L R S M K	
T-252	P D A V Y L H R I D L G P P I S L E R L D V G T N L G N A I A K L E D	>100
T-253	D A V Y L H R I D L G P P I S L E R L D V G T N L G N A I A K L E D A	>100
T-254	A V Y L H R I D L G P P I S L E R L D V G T N L G N A I A K L E D A K	>100
T-255	V Y L H R I D L G P P I S L E R L D V G T N L G N A I A K L E D A K E	85.3
T-256	Y L H R I D L G P P I S L E R L D V G T N L G N A I A K L E D A K E L	90.7
T-257	L H R I D L G P P I S L E R L D V G T N L G N A I A K L E D A K E L L	1.5
T-258	H R I D L G P P I S L E R L D V G T N L G N A I A K L E D A K E L L E	2.2
T-259	R I D L G P P I S L E R L D V G T N L G N A I A K L E D A K E L L E S	1.7
T-260	I D L G P P I S L E R L D V G T N L G N A I A K L E D A K E L L E S S	4.9
T-261	D L G P P I S L E R L D V G T N L G N A I A K L E D A K E L L E S S D	5.7
T-262	L G P P I S L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q	6.5
T-263	G P P I S L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q I	10.1
T-264	P P I S L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q I L	1.1
T-265	P I S L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q I L R	3.1
T-266	I S L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q I L R S	13.0
T-267	S L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q I L R S M	12.3
T-268	L E R L D V G T N L G N A I A K L E D A K E L L E S S D Q I L R S M K	7.3

FIG. 2. Peptide walks through the HR2 domains of RSV, HPIV-3, and MV. The amino acid sequences of the complete HR2 domains identified by the motif searches are shown in boxed areas for each virus. Sequential 35-aa peptides (shifting by 1 aa from the N termini) were synthesized to scan across the entire HR2 domain for each virus as indicated. Each peptide is designated by its compound number and the EC_{50} values (μg/ml) obtained in the antiviral screening assays of crude peptide preparations are indicated in the column at the right.

Fig. 4*B*. An HR1 peptide, T-184, from HPIV-3 (Fig. 4*A*) demonstrated helical structure in PBS similar to that demonstrated for DP-107 of HIV-1 TM (15). T-184 has the amino acid sequence: Ac-LKEAIRDTNKAVQSVQSSIGN-LIVAIKSVQDYVNK-NH_2 (aa 147–181). HR1 peptides from RSV and MV also showed evidence of helical structure in PBS. However, like DP-178 (11), none of the HR2 peptides displayed significant helical structure in PBS, but they did exhibit significant helical content when examined in a helix-inducing environment of 50% trifluoroethanol (Fig. 4*B*).

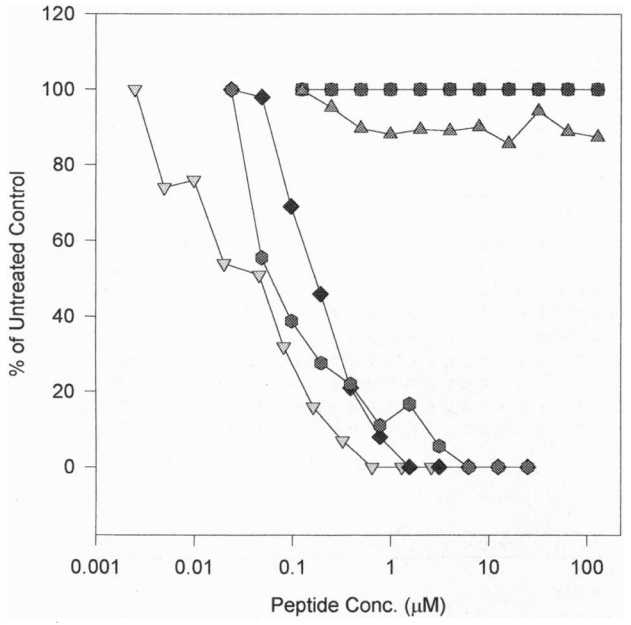

FIG. 3. Dose–response of T-118 (◆) against RSV-induced fusion, T-205 (▽) against HPIV-3-induced fusion, and T257 (●) against MV-induced fusion. Virus-infected cells (24 hr postinfection) were added to monolayers of uninfected cells in the presence of a range of peptide concentrations (μM). Cytotoxicity for T-118 (▲), T-205 (●), and T257 (■) was evaluated using the tetrazolium salt XTT in the host cell line for each virus.

DISCUSSION

We have identified peptide sequences within discrete regions of three paramyxovirus F proteins that share structural and functional features with the antiviral HIV-1 peptides DP-107 and DP-178 (11, 15, 17). The paramyxovirus F_1 protein is functionally equivalent to the TM protein of HIV-1 (Fig. 1) and is thought to play a major role in membrane fusion. A secondary structure motif searching strategy was designed to identify amino acid sequences analogous to those of DP-107 and DP-178 from the TM glycoprotein of HIV-1 (11, 15, 17) within the proteins of other enveloped viruses. This approach identified two common domains within the fusogenic proteins of a large number of enveloped viruses, including the F_1 subunits of paramyxoviruses. We chose to investigate these domains in three medically important paramyxoviruses, RSV, HPIV-3, and MV, representing three distinct genera of paramyxoviruses. The locations of these domains within the F_1 proteins relative to the fusion peptide and transmembrane anchor was conserved between HIV-1 gp41 and the paramyxovirus F_1 glycoproteins (Fig. 1). In addition, these domains overlap already identified heptad repeat regions HR1 and HR2 of paramyxoviruses (5, 9, 10, 28). Previous reports demonstrate that these domains may be critical to the fusion process of paramyxoviruses (29, 30).

Peptides were synthesized from each of the HR1 and HR2 regions of the F_1 proteins of these paramyxoviruses. Peptides from the HR1 domains exhibited several characteristics which make them similar to DP-107. For each virus tested, several of the peptides synthesized from HR1 domain exhibited helical structure in PBS (Fig. 4*A*) in a similar fashion as the HR1 analogue derived from HIV-1 (15). Peptides derived from the HR2 domains near the membrane anchor were less structured in PBS, but many of these peptides exhibited potent fusion inhibition against their homologous virus in a similar fashion as the analogous peptides from HIV-1 (11, 17).

Table 1. Selectivity of paramyxovirus and HIV-1 antiviral peptides against homologous and heterologous viruses

	Peptide EC_{50}, μM				
Virus	T-257 (MV)	T-118 (RSV)	T-205 (HPIV3)	DP-178 (HIV-1)	DP-107 (HIV-1)
MV	0.068	>25	1.19	>22	>22
HPIV3	>25	>25	0.038	>22	>22
RSV	>25	0.051	1.76	>22	>22
$HIV\text{-}1_{LAI}$	>50	>50	>50	0.0002	1.3

Although the DP-178 primary amino acid sequence is predicted by the Lupas algorithm (31) to have a high propensity to form a coiled-coil structure, under neutral pH isotonic conditions this peptide exhibited only 20% helicity by CD (data not shown). Much greater helical structure was apparent in the presence of trifluoroethanol. Similarly, the HR2 peptides derived from all three paramyxoviruses demonstrated little helical structure in PBS but significant helical content in 50% trifluoroethanol (Fig. 4*B* and data not shown). Trifluoroethanol is known to have a helix-stabilizing effect on peptides (32, 33). It has been speculated that this effect may be related to similar structures that are physiologically relevant as, for example, through association with membrane lipids (34). We have also entertained the possibility that the apparent structural transitions in the HR2 peptides according to CD analysis might be related to transitions that naturally occur in the membrane fusion process.

The fusogenic proteins of HIV, paramyxoviruses, and influenza virus have several properties in common. Proteolytic cleavage of paramyxovirus F proteins (F_0 cleaved to $F_{1,2}$) by a host cell trypsin-like protease is required for these viruses to be infectious (6, 35). Likewise, gp160 cleavage to gp120 (SU) and gp41 (TM) is required for HIV-1 to be infectious (36). The influenza virus HA also must be activated by proteolytic cleavage to $HA_{1,2}$ in order for virus to be infectious (3, 4, 16). Whereas the triggering event for induction of influenza virus fusion is a low pH shift in the endosomal compartment during infection, membrane fusion for the lentiviruses and paramyxoviruses occurs at neutral pH and events other than low pH are required to trigger conformational changes leading to fusion between virion and host cell membranes. Induction of HIV-1 TM-mediated fusion is consistent with conformational changes induced during interaction of gp120 (SU) with CD4 at the cell surface (37–39). The triggering event for paramyxoviruses has not been fully characterized (5). Similar to proposed models for influenza virus HA or HIV-1 TM, the N-terminal fusion peptide domain of the F protein is sequestered within the protein after cleavage activation and is held in a metastable conformation until the triggering event releases the fusion peptide, allowing it to interact with the host cell membrane (3, 5). Additional structural rearrangements in HR1 and HR2 domains are likely to occur during the fusion process, based on what is believed to occur within influenza HA (16, 40). The working hypothesis for the mechanism whereby these peptides block fusion is that during the course of these triggered structural rearrangements, inhibitory peptides bind to the fusion protein and block its transition to a fusogenic conformation (12). This hypothesis is supported by a recent report demonstrating that the HIV-1 DP-178 domain physically interacts with a gp41 domain which includes DP-107 (13). This result supports the notion that similar binding interactions may exist within the F proteins of paramyxoviruses. Preliminary results suggest that the HR1 and HR2 domains of RSV F protein also interact in a highly selective and specific manner (data not shown).

The peptide scanning approach used in these studies allowed us to explore the entire HR2 region of all three paramyxoviruses. Overlapping 35-aa peptides scanning the HR2 domain of each paramyxovirus F_1 glycoprotein were synthesized and tested for antiviral activity. Crude preparations of peptides representing DP-178-like domains from the F_1 subunits of

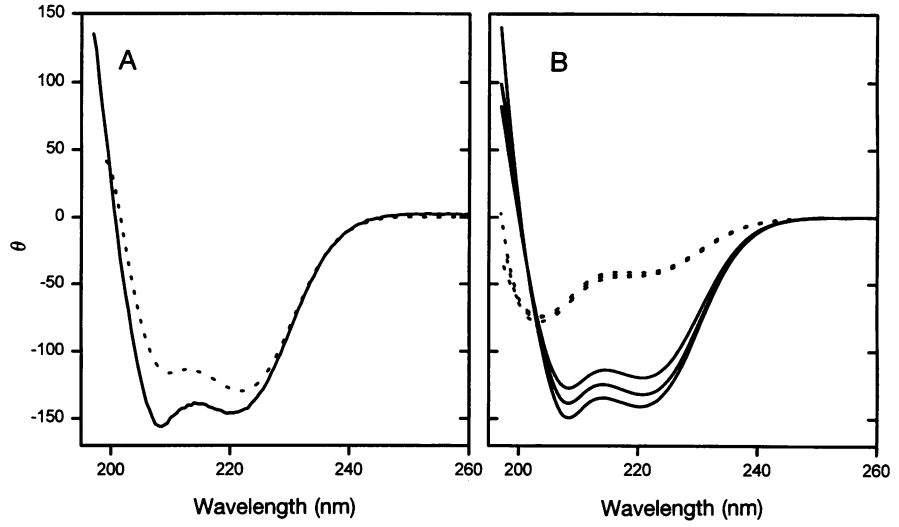

FIG. 4. (*A*) CD spectra of an HPIV-3 HR1 domain peptide, T-184, dissolved in PBS (----) or in 50% trifluoroethanol (——). (*B*) CD spectra of three representative HPIV-3 peptides, T-198, T-201, and T-205, in PBS (----) or in 50% trifluoroethanol (——). Trifluoroethanol stabilizes helical conformation in peptides with helical propensity. The observed elipticity, θ, is in units of degrees.

RSV, HPIV-3, and MV exhibited antiviral and antifusion activity specific for the virus of origin and exhibited little, if any, cytotoxic activity at 100 μg/ml.

Significantly, the antiviral activity of these peptides was specific for the virus of origin. Active RSV, HPIV-3, or MV F_1 peptides did not block HIV-1 syncytium formation at concentrations up to 50 μM (Table 1). Similarly, neither DP-107 nor DP-178 from HIV-1 gp41 blocked RSV, HPIV-3, or MV fusion. Only the HPIV-3 peptide (T-205) appeared to be somewhat crossreactive with both RSV and MV, although inhibition still required >30-fold higher concentrations compared with HPIV-3 and the peptide did not crossreact at all with HIV-1. Interestingly, the RSV and MV peptides were not active against each other or against HPIV-3 (>25 μM). None of the peptides tested exhibited sufficient cytotoxic effects to determine a CC_{50} value (>100 μM). These peptides exhibit selective indices ranging from >1900 to >3000 and, therefore, are highly selective and specific inhibitors of viral fusion and infection.

The studies of Wild *et al.* (11, 15), Carr and Kim (16) and Wiley and coworkers (40) represent the first insights into the relationship between structural domains within viral fusogenic proteins and their roles in mediating membrane fusion. In this report, we have identified domains within F_1 of the representative paramyxoviruses RSV, HPIV-3, and MV that correspond to the DP-107 (T-21) and DP-178 (T-20) domains of the HIV-1 TM protein. Consequently, the results obtained support the notion that conserved features exist between retrovirus and paramyxovirus fusion proteins (9). This information may lead to a better understanding of the similarities of structure and function of these essential viral proteins. It is likely that the HIV TM peptides and the paramyxovirus peptides identified in this study will provide a path to antiviral discovery for other fusogenic viruses such as influenza. The finding that the HR2 peptides have highly selective antiviral activity indicates that they represent important molecular targets for further antiviral drug discovery. Compounds which block these paramyxovirus targets may follow the HIV TM peptide T-20 for clinical evaluation.

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