Potent Suppression of Viral Infectivity by the Peptides that Inhibit Multimerization of Human Immunodeficiency Virus Type I (HIV-1) Vif Proteins

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Abstract

Virion infectivity factor (Vif) is essential for the replication of human immunodeficiency virus type I (HIV-1) in vivo, but its function remains uncertain. Recently, we have shown that Vif proteins are able to form multimers, including dimers, trimers, or tetramers. As the multimerization of Vif proteins is required for Vif function in the viral life-cycle, we propose that it could be a novel target for anti-HIV-1 therapeutics. Through a phage peptide display method, we have identified a set of 12-mer peptides containing PXP motif that binds to HIV-1 Vif protein. These proline-enriched peptides potently inhibited the Vif-Vif interaction in vitro. We have also screened a set of synthesized Vif peptides (15-mer), which covers all the amino acids of the HIV-1 Vif protein sequence, for their ability to inhibit the Vif-Vif interaction in vitro. We demonstrated that Vif-derived proline-enriched peptides that contain the \textsuperscript{161}PPLP\textsuperscript{164} domain, are able to inhibit the Vif-Vif interaction. Conversely, the deletion of the \textsuperscript{161}PPLP\textsuperscript{164} domain of Vif protein will significantly impair the capability of Vif proteins to interact with each other, indicating that the \textsuperscript{161}PPLP\textsuperscript{164} domain plays a key role in Vif multimerization. All these results demonstrate that the proline-enriched peptides block the multimerization of Vif through interfering the polyproline interfaces of Vif formed by \textsuperscript{161}PPLP\textsuperscript{164} domain. Moreover, these peptides that inhibit the Vif-Vif interaction in vitro potently inhibit HIV-1 replication in the “non-permissive” T-cells. We propose that this study starts a novel strategy to develop structural diverse inhibitors of Vif, such as peptidomimetics or small organic molecules.
Virion infectivity factor (Vif) protein of HIV-1 is required for viral replication in vivo (1, 2). In cell culture systems, HIV-1ΔVif viruses are incapable of establishing infection in certain cells, such as H9 T-cells, peripheral blood lymphocytes (PBLs), and monocyte-derived macrophages (3-6). HIV-1 viruses with a defective vif gene are not able to complete intracellular reverse transcription and endogenous reverse transcription in cell-free virions, when mild detergent is utilized to make the viral envelope permeable (7-10). Most studies indicated that the expression of viral components, including viral proteins and nucleic acids, are not altered in the virions produced from non-permissive cells (3, 10, 11). However, the deletion of the vif gene will result in alterations of virion morphology (12-14). Various hypotheses have been proposed regarding the molecular mechanisms of Vif protein. It has been reported that defect of vif could affect the maturation of Gag precursor (15). Further, Vif could directly bind to the protease domain of pol precursor and prevent the improper cleavage of Gag precursors before viral assembly (16). It was also proposed that Vif protein is required to counteract an unknown endogenous inhibitor (s) in the virus-producing cells (17, 18). Recent studies further indicated this endogenous inhibitor is CEM15, which is only expressed in the non-permissive cells. Introduction of CEM15 into the permissive cells will generate a non-permissive phenotype (19). However, the function of CEM15 remains unknown. As its sequence is similar with APOBEC-1(apoB mRNA editing catalytic subunit 1), a cytidine deaminase that can change cytidine into uridine in the mRNA of apolipoprotein B, CEM15 could affect the genomic RNA of HIV-1. Interestingly, we and others have shown that Vif is an RNA-binding protein and is an integral component of an mRNP complex of viral RNA (20, 21). The Vif protein in this mRNP complex may protect viral
RNA from various endogenous inhibitors and could mediate viral RNA engagement with HIV-1 Gag precursors. As such, Vif could play a key role in the proper trafficking of the viral genetic substance (genomic RNA) in the lentivirus-producing cells.

As Vif is essential for HIV-1 replication, it is an important target for anti-HIV therapeutics. However, as its molecular mechanism in viral life cycle remains to be further determined, it is quite difficult to generate small molecule inhibitor(s) to block Vif function at the present time. Recently, we have found that Vif proteins are able to form multimer (22). It is well known that multimerization is critical to the biological activity of many prokaryotic and eukaryotic proteins and is a common mechanism for the functional activation/inactivation of proteins. Therefore, multimerization has been an ideal target for the development of inhibitors of various proteins (23-25).

In this report, we demonstrate that Vif multimerization could be a promising intervention target for anti-HIV-1 agent development. We have found that a set of proline-enriched peptides is able to bind to Vif protein, inhibit the Vif-Vif interaction and inhibit viral replication in cell culture. Our data demonstrates that, although the function and structure of Vif remains uncertain, we have still successfully developed the potent Vif antagonists, based upon the biochemical characteristics of Vif protein.
Materials and Methods

Plasmid constructions, expression of GST fusion proteins, and synthesis of $^{35}\text{S}$-labeled proteins by in vitro translation

The construction of pGEX-Vif, pCITE-Vif, pCITE-Vif(Δ151-192), and pCITE-Vif(Δ151-164) were described previously (20, 22). Vif(ΔPPLP) genes were generated by polymerase chain reaction (PCR)-mediated mutagenesis, and then inserted into pGEX vector. The $vif(ΔPPLP)$ gene was also inserted into pCITE-4a vector (Novagen, Madison, WI) for in vitro translation. $^{35}\text{S}$-labeled Vif or its mutant proteins were synthesized by in vitro transcription and translation utilizing SPT3 kits (Novagen, Madison, WI) in the presence of $[^{35}\text{S}]$methionine (1,000 Ci/mmol; Amersham Pharmacia Biotech), as described previously (20). The GST, GST-Vif and other GST fusion Vif mutant proteins were produced according to the previously described methods (20, 22). The tyrosine kinase Hck genes were generated by PCR amplification and then inserted into the pGEX vector. GST-Hck fusion protein was expressed and purified with the same procedure as for GST-Vif.

Phage display peptide screening

Vif-binding peptides displayed on M13 phages were selected using the Ph.D.-12 phage display peptide library kit (New England Biolabs, Beverly, MA). Phage panning procedures were performed according to the manufacturer’s protocol with some modifications. Briefly, GST-Vif fusion protein attached to glutathione-conjugated agarose beads was used as target for phage panning. For each round of panning, $10^{11}$ phages were first absorbed with GST, followed by mixing with 3 ml GST-Vif attached to glutathione-agarose beads. After binding at room temperature for 1 hr, the GST-Vif
binding phages were then eluted by 5 mM reduced glutathione. The eluted phages were amplified by mixing the elution with 20 ml of *E. coli* ER2738 culture (O.D at 0.6). After incubation at 37 °C with vigorous shaking for 4 hrs, the bacterial cells were pelleted and the phages in the supernatant were precipitated by PEG (20%)/NaCl (2.5 M). After resuspension in TBS and re-precipitation by PEG, the phages were suspended in 200 µl TBS, 0.02%NaN₃. The titration of the eluted or amplified phages was determined by infecting the *E. coli* ER2738 mixed in the conditioned medium-agar plates, as described in the kit protocol. After 3 rounds of panning, individual phage plaques from the GST or GST-Vif elution were selected for amplification, respectively. Phage DNA was then purified and sequenced.

**Determination of binding affinity by ELISA**

An enzyme-linked immunosorbent assay (ELISA) was performed to measure the relative binding affinity of phages to GST, GST-Vif or GST-Vif (∆151-192). The protocol supplied by the manufacturer was followed. Briefly, 150 µl of 100 µg/ml GST and GST-Vif in 0.1M NaHCO₃ (pH 8.6) was coated on 96 well microtiter plates respectively and incubated at 4 °C overnight. The plates were blocked with blocking buffer [0.1M NaHCO₃, pH 8.6, 5 mg/ml bovine serum albumin (BSA)] for 2 hrs at room temperature. The individual phage clones were 4-fold-serially diluted (from 10¹¹ to 10⁵) and added to the wells coated with GST, GST-Vif, or GST-Vif (∆151-192) and incubated for 2 hrs at room temperature. After washing, HRP-conjugated anti-M13 antibody was added to bind the phages. After incubation at room temperature for 1 hr, the excess antibody was washed, the substrate was added and color development was allowed to proceed. The phages captured by Vif were therefore semi-quantitated. OD at 405 nm equal to or greater than 0.15 was considered as positive.
Peptide synthesis

HIV-1 consensus B Vif (15-mer) peptides were provided by the NIH AIDS Research & Reference Reagent Program. All the other peptides were synthesized by solid-phase techniques using a Symphony Multiplex synthesizer (Protein Technologies, Inc., Tucson, AZ) and a 9050 Peps synthesizer Plus automated peptide synthesizer (Perseptive Biosystems, Cambridge, MA) with N\textsuperscript{\textalpha}-Fmoc\textsuperscript{N-(9-fluorenyl)methoxycarbonyl}/tBu (tert-butyl) chemistry. Biotin peptides were biotinylated by Biotin (Sigma) at the N-terminus. The peptides were characterized by analytical HPLC and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). All the peptides were at least 95% pure, as determined by HPLC.

In vitro Vif-Vif /Vif-Hck interactions and their inhibition by peptides

A GST pull-down assay is used to study the in vitro protein-protein interactions. The GST-fusion proteins on agarose beads are first generated, as described previously, except without elution with 5 mM glutathione (20). The \(^{35}\text{S}\)-labeled, in vitro translated Vif proteins are then mixed with GST-fusion proteins conjugated agarose beads in washing(binding buffer [150 mM NaCl, 10 mM Tris-HCl (pH8.0), and 0.1% Triton-X-100]. Binding is allowed to proceed at 23 °C for 20 mins, and then at 4 °C for 1 hr. For the inhibition of Vif-Vif/Vif-Hck binding by peptides, \(^{35}\text{S}\)-labeled Vif proteins were added to GST-Vif/GST-Hck conjugated agarose beads and incubated with peptides at different concentrations in binding buffer at 4 °C for 1 hr. The beads were then washed with washing(binding buffer 3 times and the bead-bound \(^{35}\text{S}\)-labeled Vif proteins were fractionated by SDS-PAGE, followed by autoradiography and quantitated using a PhosphorImager (Molecular Dynamics, Sunnyview, CA).
Peptide internalization experiment

H9 cells were suspended in serum-free RPMI-1640 supplemented with 4 mM L-glutamine and incubated with the peptides for 30 min. After washing three times with phosphate-buffered saline (PBS), the cells were fixed with 4% formaldehyde in PBS for 10 min at room temperature. Cells were then washed twice with PBS, and treated with 0.1% Triton X-100 in PBS for 10 min. After additional two washes with PBS, cells were incubated with blocking buffer (3% BSA in PBS) for 1 hr at room temperature, followed by incubation with streptavidin-FITC(Sigma, MO) at 2 µg/ml in blocking buffer for 5-10 min in the dark. Cells were then washed with PBS and cell suspensions were smeared on glass microscope slides for fluorescence microscopy using an Olympus BX60 fluorescence microscope.

Viral infectivity assay

H9 cells (1x10^6) were mixed with HIV-1_{NL4-3} viruses at a MOI 0.01. After incubation at 37 °C for 5 hrs, the excess viruses were removed and the cells were cultured in the presence of RPMI-1640 medium plus 10% fetal bovine serum, with or without peptides at a concentration of 50 µM. Every 3-4 days, the supernatants were harvested and refreshed. The effects of these peptides upon viral infectivity were monitored by detecting the HIV-1 p24 antigen level in the cell culture supernatant via ELISA, as described previously (20, 26, 27)
Results

Identification of PXP motif-containing peptides binding to Vif protein

In order to search for the peptides that bind with HIV-1 Vif protein, the phage peptide display method was employed. The procedures described in the manual supplied by manufacturer were followed. After three-rounds of panning, the phage displayed peptides that bind with GST-Vif, were identified by sequencing DNA in the knot region of the phages. Through these methods, we have identified a set of 12-mer peptides containing a PXP motif that bind to the Vif protein (Table I).

To determine the binding affinity of various PXP motif-containing peptides to Vif protein, a simple assay based upon an ELISA was used to determine the relative affinity. The phages at various concentrations captured by Vif were semi-quantitated. Fig. 1A demonstrates that, among PXP motif containing peptides, VMI 5, VMI 7, VMI 9, and VMI 16 bind to Vif at the highest affinities. The C-terminus-deleted Vif protein binds with PXP motif containing peptides at low affinity, indicating that PXP motif containing peptides bind to Vif protein through the C-terminus of the Vif protein.

PXP motif-containing peptides inhibit Vif-Vif interaction in vitro

We noted that Vif proteins of various HIV-1 strains all contain proline-rich sequence at their C-terminus (^{161}PPLP^{164} in NL4-3 strain), and we have demonstrated that the proline-enriched domain (^{151}AALIKPKQIKPPLP^{164}) is required for Vif multimerization (22). Therefore, it is interesting to examine whether PXP motif-containing peptides inhibit Vif-Vif interaction. To this end, some of these peptides containing the PXP domain, identified from phage display libraries, were chemically synthesized and examined for their ability to inhibit Vif-Vif binding. Fig. 1B indicates that peptides containing the PXP motif, such as SNQGGSPLPRSV(VMI 7) or
LPLPAPSFHRTT (VMI 9), could significantly inhibit Vif-Vif interaction. The IC$_{50}$ for the inhibition of Vif multimerization is 7.43 µM for VMI 7 and 4.84 µM for VMI 9, respectively (Fig. 1B). A Vif derived 12-mer peptide, $^{155}$KPKQIKPPLPSV$^{166}$[Vif(155-166)], which is originated from the proline-enriched C-terminus of Vif, also has the similar inhibition activity upon Vif-Vif interaction (IC$_{50}$= 17.39µM).

We have also screened a set of synthesized Vif peptides (15 mer), which includes all the amino acids of HIV-1 Vif protein, for their ability to block the Vif-Vif interaction in vitro. We demonstrated that proline-enriched Vif peptides, such as $^{153}$LITPKKKIKPPLPSVT$^{167}$, $^{157}$KKIKPPLPSVTKL$^{171}$, which contain the 161PPLP$^{164}$ domain, are able to inhibit the Vif-Vif interaction significantly, further supporting that PXP motif-containing peptides inhibit Vif multimerization (Fig. 1B and Fig.2).

Conversely, this result also suggests that the 151-165 region of Vif is responsible for Vif-Vif binding. The peptides, derived from region of 145-163 which is upstream of the 161PPLP$^{164}$ domain, are also able to moderately inhibit Vif-Vif interaction, suggesting that the amino acid residues at this region could also participate in the Vif-Vif interaction.

The 161PPLP$^{164}$ domain is required for Vif multimerization

As the PXP motif is also shared by Vif in the 161PPLP$^{164}$ domain that is located within the putative Vif multimerization domain, and the PXP motif-containing peptides are able to inhibit Vif-Vif interaction, it is interesting to investigate whether the 161PPLP$^{164}$ domain is required for Vif-Vif interaction. To this end, site-directed mutagenesis was performed to delete 161PPLP$^{164}$, $^{151}$AALIKPKQIKPPLP$^{164}$, and the Vif C-terminus (151-192). The mutants were expressed with an in vitro translation system, in the presence of $^{35}$S-methionine, or expressed as GST-fusion proteins. The $^{35}$S-labelled Vif or Vif mutants were then bound with GST-Vif or GST-Vif (∆PPLP) that were
conjugated with glutathione-coated agarose beads. As described previously, Vif mutant proteins deleted at the C-terminus (151-192) or \textsuperscript{151}AALIKKKIKPPLP\textsuperscript{164} have decreased binding to Vif (Fig. 3)(22). Vif mutant protein just deleted at the \textsuperscript{161}PPLP\textsuperscript{164} domain also showed a decrease in its binding to Vif. Interestingly, the protein-protein interactions between the Vif mutants deleted at \textsuperscript{161}PPLP\textsuperscript{164} domain were significantly decreased (Fig. 3). These data indicated that the \textsuperscript{161}PPLP\textsuperscript{164} domain is required for Vif-Vif multimerization.

**PXP motif containing peptides inhibit Vif-Hck binding**

It has been demonstrated that Hck kinase can also bind with Vif through the \textsuperscript{161}PPLP\textsuperscript{164} domain (28). It is possible that PXP motif containing peptides are able to block the interaction between Vif and Hck and other protein kinases. To this end, \textsuperscript{35}S-labeled Vif was allowed to bind with GST-Hck, in the presence or absence of various peptides. As described by others, Vif is able to bind with Hck. In the presence of VMI 7, VMI 9 and Vif(155-166), the binding between Vif and Hck is significantly decreased (Fig. 4).

**PXP motif-containing peptides inhibit HIV-1 replication**

To examine the inhibitory effects of PXP motif containing peptides upon viral infectivity in cell culture, the peptides must be introduced into the virally-infected cells by a reliable method. As antennapedia homeodomain (Ant, RQIKIWFQNRRMKWKK) has been widely used to effectively carry peptides into various living cells (29-34), Ant fusion peptides, Ant-VMI 7, Ant-VMI 9 and Ant-Vif(155-166), were synthesized and their activity for in vivo inhibition of HIV-1 replication were investigated. These peptides did not show any toxicity to H9 cells at the concentration of 50 µM (data not
shown). To examine whether these fusion peptides are able to enter the H9 cells, the cell permeability of biotin Ant-VMI 9 was determined. Fig. 5A indicates that the Ant fusion peptide can efficiently enter the H9 cells and locate in the cytoplasm. As Vif mainly locates in the cytoplasm of virus-infected cells, the fusion peptide should physically interact with Vif protein.

The fusion peptides were then added into the cell culture to examine their capability to inhibit HIV-1 replication. H9 cells, a non-permissive cell line that requires Vif to counteract the endogenous inhibitor, were infected with HIV-1 viruses, in the presence or absence of various fusion peptides. At the concentration of 50 µM, the fusion peptides, Ant-VMI 7, Ant-VMI 9, and Ant-Vif (155-166) are able to effectively inhibit HIV-1 replication. As a control, the Ant peptide itself does not have any anti-HIV-1 activity (Fig. 5B).
Discussion

We have demonstrated that the $^{151}$AALIKPKQIKPPLP$^{164}$ domain of HIV-1 Vif is critical for Vif multimerization, which is required for Vif function (22). In this report, we have further demonstrated that the $^{161}$PPLP$^{164}$ domain plays a key role in Vif-Vif interaction. Our current results suggest that Vif-Vif binding occurs, at least in part, through the direct interaction between $^{161}$PPLP$^{164}$ domains in each Vif molecule. As the function of Vif remains unknown, it is difficult to investigate the molecular mechanism regarding how Vif multimerization is required for Vif function. However, recent studies indicated that Vif is required to counteract the endogenous inhibitor CEM15, which is a putative cytidine deaminase (19, 35). As Vif binds to HIV-1 RNA, it is reasonable to assume that Vif-RNA binding could protect the HIV-RNA from RNA editing (20, 21). If so, Vif-RNA binding could be the major mechanism for Vif function. It is therefore quite important to study the correlation between Vif-RNA binding and Vif-Vif interaction. As Vif binds to RNA through its N-terminus while Vif-Vif interaction takes place at the C-terminus, Vif-Vif interaction could be correlated with Vif-RNA binding. Conversely, Vif is able to bind with Gag protein through the positive-charged amino acids in the 151-164 region at the C-terminus, and Vif binds to Hck also through the $^{161}$PPLP$^{164}$ domain. Therefore, the Vif-Vif interaction could be reversibly correlated with Vif-Gag binding or Vif-Hck binding (13, 28). These hypotheses remain to be fully tested.

Through screening phage display peptide libraries, a set of proline-enriched peptides binding to Vif were identified and are able to block the Vif-Vif interaction. The proline-enriched sequence is a hydrophobic region and usually binds to the hydrophobic interface of SH3/WW domains in protein-protein interactions (36). Vif-Vif interaction
could occur between the two $^{161}\text{PPLP}^{164}$ domains or the $^{161}\text{PPLP}^{164}$ domains and other regions in the Vif protein. It seems that the PXP motif containing peptides mimic the hydrophobic structure of the $^{161}\text{PPLP}^{164}$ domain and bind to the hydrophobic interface of Vif, which is quite critical for Vif multimerization. Among these proline-enriched peptides, the peptides containing the PXPXP motif have the higher binding affinity to Vif protein. We have also tested the synthesized peptides derived from the Vif protein upon Vif-Vif interaction. Our data demonstrated that the peptides containing the $^{161}\text{PPLP}^{164}$ domain are able to inhibit Vif-Vif interaction, indicating that the $^{161}\text{PPLP}^{164}$ domain plays a key role in Vif-Vif interaction.

In this report, we demonstrated that proline-enriched PXP motif containing peptides not only inhibit Vif-Vif interaction, but also the binding between Vif and Hck. It is notable that the PXP motif containing peptides have been shown to inhibit the activation of various SH3 domain-contained protein kinases (36, 37). As the peptides identified in this report do not have any toxicity to the cultured cells at concentrations used to inhibit HIV-1 replication, they should have certain specificity in blocking Vif-Vif or Vif-Hck interactions, rather than inhibiting the activation of other protein kinases used in maintaining the normal functions of the cells.

As Vif is required for HIV-1 replication and Vif multimerization is important for the function of Vif, inhibitor(s) that block the formation of Vif multimer should inhibit HIV-1 replication. A reliable method was used to allow the peptides that inhibit Vif-Vif interaction to effectively enter HIV-1-infected cells. Indeed, the peptides that effectively inhibit Vif-Vif interaction potently inhibit HIV-1 replication in cell culture (Fig. 5B).
In this report, we have shown that the $^{161}$PPLP$^{164}$ domain of Vif is a valuable target for developing Vif inhibitors. As the PXP motif containing peptides potently inhibit Vif-Vif interaction and inhibit HIV-1 replication in non-permissive cells, it is interesting to further investigate the structural mechanisms of these peptide inhibitors and develop more potent nonpeptide Vif inhibitors, such as peptidomimetic or small organic molecular inhibitors. Because of the essential role of Vif in HIV-1 replication, we believe that the development of these Vif inhibitors may represent a new strategy for anti-AIDS therapy (38, 39).
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Figure Legends

**Fig. 1A. Relative affinity comparisons between PXP motif containing peptides.** GST fusion protein (100 µg/ml) of Vif, Vif (Δ151-192), and GST only were coated onto the 96 well plate. The phages clones isolated through GST-Vif-containing column were serially diluted and added. After incubation to allow phage-Vif binding, excess phages were washed off. Anti-M13 phage antibody, conjugated with HRP, was added to bind the phages that were captured by GST-Vif. After washing, the substrate was added and color development was allowed to proceed. The phages captured by GST-Vif were therefore semi-quantitated. OD at 405 nm equal to or greater than 0.15 was considered to be positive. The phage sample number (VMI) is the same as shown in Table 1. **1B. In vitro binding affinity of peptides to Vif.** Various Peptides (10^{-7}, 5 \times 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M) were added to the mixture of 35S-labeled Vif and GST-Vif conjugated agarose beads. The 35S-labeled Vif binding to GST-Vif were dissociated from beads by adding 2% SDS loading buffer and then analyzed by SDS-PAGE, followed by autoradiography and quantitation using PhosphorImager. IC_{50} is the concentration of the peptides inhibiting 50% of 35S-labeled Vif binding to GST-Vif in GST pull-down assays.

**Fig. 2. The inhibition of HIV-1 Vif (15-mer) peptides upon Vif-Vif binding.** HIV-1 consensus B Vif (15-mer) peptides (100µM) were added to the mixture of 35S-labeled Vif and GST-Vif conjugated agarose beads. The 35S-labeled Vif binding to GST-Vif were dissociated from beads by adding 2% SDS loading buffer and then analyzed by SDS-PAGE, followed by autoradiography and quantitation using a PhosphorImager.
**Fig. 3. Deletion of PPLP eliminates Vif-Vif interaction.** GST-Vif or GST-Vif(ΔPPLP) conjugated agarose beads were mixed with $^{35}$S-labeled Vif or its mutants in binding buffer and incubated at 4 °C for 1 hr. The $^{35}$S-labeled Vif or its mutants remaining on beads were dissociated from beads by adding 2% SDS loading buffer and then analyzed by SDS-PAGE, followed by autoradiography and quantitation using a PhosphorImager. A: GST-Vif/$^{35}$S-Vif; B: GST-Vif/$^{35}$S-Vif(Δ151-192); C: GST-Vif/$^{35}$S-Vif(Δ151-164); D: GST-Vif/$^{35}$S-Vif(ΔPPLP); E: GST-Vif(ΔPPLP)/$^{35}$S-Vif; F: GST-Vif(ΔPPLP) /$^{35}$S-Vif(ΔPPLP).

**Fig. 4. The *in vitro* inhibition by the peptides on Vif-Vif or Vif-Hck binding.** Various peptides (100µM) were added to the mixtures of $^{35}$S-labeled Vif and GST-Vif or GST-Hck conjugated agarose beads. The $^{35}$S-labeled Vif binding to GST-Vif or GST-Hck were dissociated from beads by adding 2% SDS containing loading buffer and then analyzed by SDS-PAGE, followed by autoradiography and quantitation using a PhosphorImager.

**Fig. 5. (A). Internalization of peptides.** H9 cells were incubated with biotinylated peptides VMI 9 and Ant-VMI 9 for 30 min. The excess peptides were then washed off. After fixing, the internalized peptides were detected with streptavidin-FITC, followed by visualization with fluorescence microscopy. A: Ant-VMI 9, fluorescence; B: Ant-VMI 9, phase-contrast; C: VMI 9, fluorescence; D: VMI 9, phase contrast (B). **Ant fusion peptides inhibit HIV-1 replication.** H9 cells were infected by HIV-1NL4-3 virions at 37°C for 4 hrs. The infected H9 cells (1x10^6) were then cultured in duplicate, in 2 ml of RPMI-1640 medium plus 10% fetal bovine serum without or with peptides (50 µM).
Portions of the supernatants (0.5 ml) were collected every 3 to 4 days. The HIV-1 p24 antigen levels were determined by ELISA. This data represents three independent experiments.
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Table 1  PXP motif containing peptides identified by phage display peptide screening

| Sequence ID | Peptides       |
|-------------|----------------|
| VMI1        | SNFASITTPRPH   |
| VMI2        | WPTNPTTVPVPS   |
| VMI3        | LTSDTYFLPVPA   |
| VMI4        | SLHWPVSHPPPP   |
| VMI5        | SVSVGMKSPRPP   |
| VMI6        | WHSQRLSPVPPA   |
| VMI7        | SNQGSSPLPRSV   |
| VMI8        | SEPHLPFPVLPH   |
| VMI9        | LPLAPSFHRTT    |
| VMI10       | YPLHPMWSMLP    |
| VMI11       | TMTPPPTSVRGT   |
| VMI12       | TPLPTIRGDTGT   |
| VMI13       | GPPPHHRDYHGP   |
| VMI14       | YPAPIKVLLPNS   |
| VMI15       | SYPMAFPLHNN    |
| VMI16       | SYPWSWSTPAGR   |
Fig. 1A
Fig. 1B

Inhibition of Vif-Vif binding (%) vs. Peptide concentrations (M)

- VMI 7
- VMI 9
- Vif(155-166)
Fig. 2

HIV-1 Consensus B Vif (15-mer) Peptide

ViF-ViF Binding (%)
Fig. 3
Fig. 4
Fig. 5A
Fig. 5B
Potent suppression of viral infectivity by the peptides that inhibit multimerization of human immunodeficiency Virus type I (HIV-1) Vif proteins
Bin Yang, Ling Gao, Lin Li, Zhixian Lu, Xuejun Fan, Charvi A. Patel, Roger J. Pomerantz, Garrett C. DuBois and Hui Zhang

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