A Novel Peptide Antagonist of CXCR4 Derived from the N-Terminus of Viral Chemokine vMIP-II†

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ABSTRACT: The viral macrophage inflammatory protein-II (vMIP-II) encoded by Kaposi's sarcoma-associated herpesvirus is unique among all known chemokines in that vMIP-II shows a broad-spectrum interaction with both CC and CXC chemokine receptors including CCR5 and CXCR4, two principal coreceptors for the cell entry of human immunodeficiency virus type 1 (HIV-1). To elucidate the mechanism of the promiscuous receptor interaction of vMIP-II, synthetic peptides derived from the N-terminus of vMIP-II were studied. In contrast to the full-length protein that recognizes both CXCR4 and CCR5, a peptide corresponding to residues 1−21 of vMIP-II (LGASWHRPDKCCLGYQKRPLP) was shown to strongly bind CXCR4, but not CCR5. The IC_{50} of this peptide in competing with CXCR4 binding of ^{125}I-SDF-1α is 190 nM as compared to the IC_{50} of 14.8 nM of native vMIP-II in the same assay. The peptide selectively prevented CXCR4 signal transduction and coreceptor function in mediating the entry of T- and dual-tropic HIV-1 isolates, but not those of CCR5. Further analysis of truncated peptide analogues revealed the importance of the first five residues for the activity with CXCR4. These results suggest that the N-terminus of vMIP-II is essential for its function via CXCR4. In addition, they reveal a possible mechanism for the distinctive interactions of vMIP-II with different chemokine receptors, a notion that may be further exploited to dissect the structural basis of its promiscuous biological function. Finally, the potent CXCR4 peptide antagonist shown here could serve as a lead for the development of new therapeutic agents for HIV infection and other immune system diseases.

Chemokines are a superfamily of small proteins of pro-inflammatory mediators and potent chemoattractants for T cells, monocytes, and macrophages. Based on the positions of two conserved cysteine residues in their N-termini, chemokines can be mainly divided into CC and CXC subfamilies (*1*). Chemokine receptors play an important role as coreceptors for the entry of HIV-1[1] into the target cell, among which CCR5 and CXCR4 are the two major HIV-1 coreceptors (*2*). Human CC chemokines such as RANTES and MIP-1β (*3*) and CXC chemokines such as SDF-1 α (*4, 5*) inhibit HIV-1 entry via CCR5 and CXCR4 receptors, respectively. In general, a particular chemokine can only bind one or more receptors within the same subfamily. However, vMIP-II, a chemokine encoded by human herpesvirus 8 (HHV-8) (*6*), displays diverse interactions with both CC and CXC chemokine receptors and inhibits HIV-1 entry mediated through CCR3, CCR5, and CXCR4 (*7, 8*). The broad-spectrum receptor binding property of vMIP-II is unique among all known chemokines and thus provides a useful template to study chemokine ligand−receptor interaction and design novel small-molecule anti-HIV agents. An important question regarding the mechanism of action of vMIP-II is whether it uses common regions for the general binding of multiple receptors or distinctive sites within vMIP-II have been evolved for the selective interaction with different receptors.

In this study, we employed a synthetic peptide approach to probe the mechanism of the biological function of vMIP-II. Comparison of amino acid sequences of vMIP-II and other human chemokines reveals that the N-terminus of vMIP-II has little homology with either CC or CXC chemokines whereas other regions of vMIP-II share a high sequence similarity with CC chemokines such as MIP-1α and MIP-1β (*8*). It is known that the N-termini in a number of other chemokines are critical for biological function (*9*). Thus, it is conceivable that the unique N-terminal sequence of vMIP-II may confer biological function distinct from other chemokines. To test this hypothesis, a synthetic peptide derived from the N-terminus of vMIP-II was synthesized and studied in various biological assays. The peptide, designated as V1, contains the amino acid sequence of residues 1−21 of vMIP-II (LGASWHRPDKCCLGYQKRPLP). This peptide displayed antagonistic activity against CXCR4, but not CCR5, and selectively inhibited CXCR4-mediated T- and dual-tropic HIV entry. The implication of these results in

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[1] Abbreviations: vMIP-II, viral macrophage inflammatory protein-II; HIV-1, human immunodeficiency virus type 1; MIP-1α, macrophage inflammatory protein 1α; FACS, fluorescence-activated cell sorter; SDF-1, stromal cell derived factor-1; RANTES, regulated upon activation, normal T cell expressed and secreted; Fmoc, *N*-(9-fluorenyl)-methoxycarbonyl.

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understanding the functional determinants of vMIP-II for interactions with chemokine receptors and developing novel anti-HIV agents is discussed.

EXPERIMENTAL SECTION

Materials. Recombinant human chemokines SDF-1, MIP-1β, and vMIP-II (R&D Systems, Minneapolis, MN) were lyophilized and dissolved as 1 μg/μL or 2.5 μg/μL stock solutions in sterile phosphate-buffered saline (PBS) and stored at -20 °C in aliquots. The radioiodinated SDF-1α and MIP-1β were purchased from DuPont NEN. The specific activities of ^{125}I-SDF-1α and ^{125}I-MIP-1β were 2200 Ci/mmol. Cell culture media and G418 were purchased from Life Technologies, Inc. The anti-CXCR4 monoclonal antibody (mAb) 12G5 (*10*) was purchased from PharMingen (San Diego, CA). 293 and NIH/3T3 cells were kindly provided by Robert W. Doms of University of Pennsylvania and maintained in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. The human pcCXCR4 and recombinant vaccinia viruses encoding two Envs of HIV-1, vSC60 (BH10) (S. Chakrabarti and B. Moss, personal communication) and vBD3 (89.6), and T7 RNA polymerase, vTF1.1 (*11*), were also generous gifts from Robert W. Doms.

Peptide Synthesis. The peptides were prepared by solid phase synthesis using Fmoc-strategy on a 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and a 9050 Pepsynthesizer Plus (Perseptive Biosystems, Cambridge, MA), as described previously (*12*, *13*). The side chain protecting groups of N$^{\alpha}$-Fmoc [*N*-(9-fluorenyl)methoxycarbonyl] amino acids were Arg, Pmc; Asp, OtBu; Cys, Trt; Gln, Trt; His, Trt; Lys, Boc; Ser, tBu, Tyr, tBu; and Trp, Boc (Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, OtBu = *tert*-butyl ester, Trt = trityl, Boc = *tert*-butyloxycarbonyl, and tBu = *tert*-butyl ester). In every coupling reaction step, a 4-fold excess of N$^{\alpha}$-Fmoc amino acid, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, and 1-hydroxybenzotriazole and a 10-fold excess of diisopropylethylamine were used. The cleavage of peptides from the resin was carried out with the cleavage reagent (trifluoroacetic acid/thioanisole/phenol/water/ethanedithiol/triisopropylsilane, 81.5:5:5:5:2.5:1) for 2 h at room temperature with gentle stirring. Crude peptides were precipitated in ice-cold methyl *tert*-butyl ether, centrifuged, and lyophilized. The crude peptides were then purified by preparative HPLC using a Dynamax-300 Å C_{18} 25 cm × 21.4 mm i.d. column with two solvent systems of 0.1% TFA/H_2O and 0.1% TFA/acetonitrile. Fractions containing the appropriate peptide were pooled together and lyophilized. The purity of the final product was assessed by analytical reverse phase high performance liquid chromatography, capillary electrophoresis, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. All peptides were at least 95% pure.

Flow Cytometry. Sup T1 cells (2×10^5) were washed with FACS buffer (0.5% bovine serum albumin, 0.05% sodium azide in PBS) and incubated with anti-CXCR4 monoclonal antibody (mAb) 12G5 (10 μg/mL) for 30 min at 4 °C. After being washed with FACS buffer, cells were incubated with 10 μg of FITC-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Inc., Birmingham, AL) for 30 min at 4 °C. After washing twice with FACS buffer, cells were fixed in the fixing buffer (2% palaformaldehyde in PBS) and then analyzed on a FACScan flow cytometer (Coulter EPICS Elite, Coolten Corp., Hialeah, FL).

^{125}I-SDF-1α Competitive Binding to CXCR4. CEM-T4 cells were harvested and washed twice with PBS. Competition binding experiments were performed using a single concentration (0.2 nM) of ^{125}I-SDF-1α in the presence of increasing concentrations of unlabeled ligands in a final volume of 100 μL of binding buffer (50 nM HEPES, pH 7.4, 1 nM $CaCl_2$, 5 nM $MgCl_2$, 0.1% bovine serum albumin) containing 2×10^5 cells. Nonspecific binding was determined by the addition of 100 nM unlabeled SDF-1α Samples were incubated for 60 min at room temperature. The incubation was terminated by separating the cells from the binding buffer by centrifugation and washing once with 500 μL of cold binding buffer. Bound ligands were quantitated by counting γ emissions.

^{125}I-MIP-1β Competitive Binding to CCR5. Following a similar experimental procedure as described above, 293 cells transfected with CCR5 and ^{125}I-MIP-1β were used to determine the specific binding activity of peptides to CCR5.

Gene Reporter Fusion Assay. Following a modified procedure published by our lab (*14*) and others (*15*−*17*), a gene reporter fusion assay was used to determine the inhibition of the peptides on the coreceptor activity of CXCR4 and CCR5 in mediating HIV-1 viral entry. HIV-1 Env proteins and T7 RNA polymerase were introduced into effector 293 cells by infection with recombinant vaccinia virus and incubated overnight at 32 °C in the presence of rifampicin (100 μg/mL). NIH/3T3 target cells were cotransfected in 6-well plates with plasmids encoding CD4, CXCR4, or CCR5 and luciferase under the control of T7 promotor by $CaPO_4$ transfection and incubated at 37 °C overnight. To initiate fusion, 10^5 effector cells were added to each well and incubated at 37 °C in the presence of ara-C and rifampicin. After 5 h of fusion, cells were lysed in 150 μL of reporter lysis buffer (Promega) and assayed for luciferase activity by using commercially available reagents (Promega).

Intracellular Calcium Measurement. Sup T1 cells and CCR5-transfected 293 cells were used to measure the intracellular calcium influx. $[Ca^{2+}]_i$ was measured using excitation at 340 and 380 nm on a fluorescence spectrometer (Perkin-Elmer LS50). Calibration was performed using 10% Triton X-100 for total fluorophore release and 0.5 M EGTA to chelate free Ca^{2+}. Intracellular Ca^{2+} concentrations were calculated by using the fluorescence spectrometer measurement program.

Chemotaxis. Migration of Sup T1 cells was assessed in disposable Transwell trays (Costar, Cambridge, MA) with 6.5 mm diameter chambers and a membrane pore size of 3 μM. SDF-1 at 100 nM (kindly provided by Elias Lolis of Yale University) in 0.5% BSA RPMI 1640 was added to the lower well. Then 100 μL of Sup T1 cells at 1×10^7 cells/mL in the same medium without SDF-1 was added to the upper well. For peptide inhibition experiments, the cells were preincubated with various concentrations of the peptide for 15 min at 25 °C. The peptide at the same concentration was also added to the lower well. After incubation at 37 °C and 5% CO_2 for 4 h, cells that migrated to the lower well were counted. For each sample, at least three independent experiments were carried out.

Table 1: Sequences of vMIP-II and N-Terminal Peptides of vMIP-II

VMIP-II (*6*)	LGASWHRPDKCCLGYQKRPLPQVL LSSWYPTSQLCSKPGVIFLTKRGRQ VCADKSKDWVKKLMQQLPVTAR
V1 (residues 1−21 of vMIP-II)	LGASWHRPDKCCLGYQKRPLP
V2 (residues 6−18 of VMIP-II)	HRPDKCCLGYQKR
V3 (residues 1−10 of vMIP-II)	LGASWHRPDK

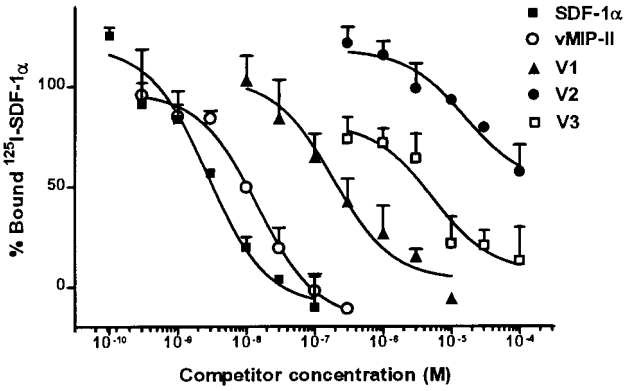

FIGURE 1: CXCR4 binding of peptides V1 (▲), V2 (●), and V3(□) as well as SDF-1α (■) and vMIP-II (○) as characterized by ^{125}I-SDF-1α competitive binding assay. The results shown here are the mean values of three independent assays. Data were processed by using Prism 2.01 (Graphpad Software, Inc.). The mean values of three independent experiments are shown.

RESULTS

The V1 Peptide Binds CXCR4 but Not CCR5. The V1 peptide was synthesized corresponding to residues 1−21 of the N-terminal region of vMIP-II (Table 1). Since vMIP-II can interact with CXCR4 and CCR5 (*8*), we tested the binding activity of V1 peptide with both receptors. For CXCR4 binding, the peptide, together with native vMIP-II and SDF-1α as controls, was examined by using both ^{125}I-SDF-1α and anti-CXCR4 mAb 12G5 competitive binding assays (Figure 1 and Table 2). The V1 peptide was shown to strongly compete with the CXCR4 binding of ^{125}I-SDF-1α in a concentration-dependent manner with an IC_{50} of 190 nM. Thus, the V1 peptide appears to have much higher CXCR4 binding affinity than other reported peptides derived from the SDF-1 N-terminus (*18, 19*). Since the dimerization of a cysteine-containing peptide derived from the SDF-1 N-terminus has been reported to contribute to receptor binding (*18*), we wanted to examine any dimer formation of the V1 peptide which contains two cysteines. Analysis by mass spectrometry demonstrated a pure monomer with no dimer detectable (data not shown), thus excluding the contribution of dimerization to the strong CXCR4 binding by the V1 peptide.

To further characterize residues within the N-terminus of vMIP-II important for CXCR4 recognition, truncated V1 analogues were synthesized (Table 1). The V2 peptide (residues 6−18 of vMIP-II) containing truncation on both ends of V1 showed a significant loss in CXCR4 binding whereas the V3 peptide (residues 1−10 of vMIP-II) containing the first half of V1 sequence retained some activity (Figure 1). The interaction of these peptides with CCR5 receptor was tested in a competitive binding assay using radiolabeled MIP-1β. All peptides did not show any binding activity with CCR5 (data not shown). These results demonstrated that the peptides derived from the N-terminus of vMIP-II interact with CXCR4 but not CCR5. This is in contrast with native vMIP-II that can recognize both receptors.

Table 2: CXCR4 Receptor Binding Affinity of vMIP-II-Derived Peptides

peptides or chemokines	IC_{50} as characterized by ^{125}I-SDF-1α (nM)	IC_{50} as characterized by 12G5 antibody (nM)
SDF-1α	2.7	19.3
vMIP-II	14.8	3.0
V1	190	640
V2	>100000	>100000
V3	5290	>10000

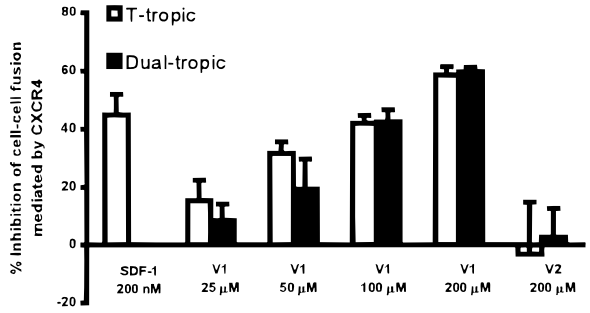

FIGURE 2: Inhibition by vMIP-II-derived peptides of HIV-1 coreceptor function of CXCR4 for vSC60 (BH10) T-tropic and 89.6 dual-tropic isolates in a cell−cell fusion assay. The bars represent the mean values of at least three independent assays, whereas the error bars are the standard errors (±SE).

The V1 Peptide Selectively Inhibits T- and Dual-Tropic HIV-1 Entry. Using a cell−cell fusion assay, we tested the ability of the peptides in blocking coreceptor function of CXCR4 and CCR5 in mediating cell entry of various HIV-1 isolates. The V1 peptide showed inhibition of both T- and dual-tropic HIV-1 gp120-mediated cell−cell fusion via CXCR4 (Figure 2). As expected from its significant loss in CXCR4 binding (Figure 1), the truncated V2 peptide did not show any activity. On the other hand, both V1 and V2 peptides displayed no effect on M-tropic HIV-1 gp120-mediated cell−cell fusion via CCR5 (data not shown). These results were consistent with binding studies and demonstrated that the V1 peptide selectively inhibited CXCR4 coreceptor function in mediating HIV-1 entry.

The V1 Peptide Blocks the Signaling and Chemotaxis of SDF-1 via CXCR4. As the V1 peptide can bind CXCR4 receptor, its ability to induce a signal or interfere with SDF-1 signaling via CXCR4 was studied by measuring intracellular calcium influx in Sup T1 cells expressing the receptor. At various concentrations, the peptide did not show any signaling activity via CXCR4, suggesting that it is an antagonist (Figure 3a). In addition, this peptide interfered with the signaling of SDF-1, a natural CXCR4 ligand, and almost completely blocked the SDF-1 signal at a concentration of 200 μM (Figure 3a). The effect of the V1 peptide on signal transduction via CCR5 was also tested in 293 cells transfected with CCR5. As expected from its lack of binding to CCR5, the peptide neither displayed signaling activity nor blocked the signal induced by MIP-1β via CCR5 (Figure 3b). The V2 peptide, which does not bind CXCR4 or CCR5 (Figure 1), did not show any effect on CXCR4 or CCR5 signal transduction (data not shown). In addition to calcium

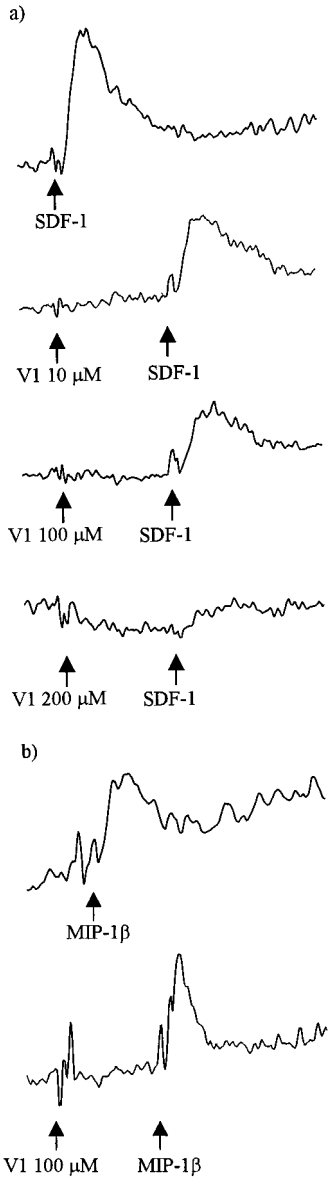

FIGURE 3: Intracellular calcium influx in Sup T1 (a) and CCR5 transfected 293 cells (b). The V1 peptide with the indicated concentrations and SDF-1 (100 nM) or MIP-1β (100 nM) were sequentially used to treat Sup T1 and 293 cells, respectively.

influx, the V1 peptide was tested in assays of chemotaxis of Sup T1 cells. Consistent with its ability to interfere with SDF-1 signaling via CXCR4, the V1 peptide was found to inhibit the chemotactic activity of SDF-1 in a concentration-dependent manner (Figure 4).

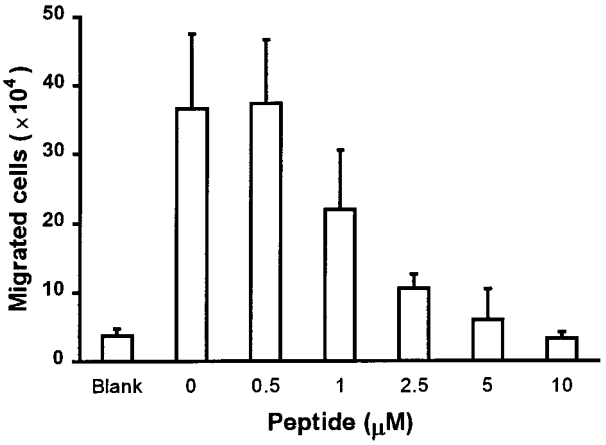

FIGURE 4: Inhibition by the V1 peptide of chemotaxis of Sup T1 cells induced by SDF-1. The bars represent the mean values of three independent assays, whereas the error bars are the standard errors (±SE).

DISCUSSION

The viral chemokine vMIP-II differs from all known human chemokines in that vMIP-II binds with high affinity to a number of both CC and CXC chemokine receptors (*8*). This unique property of vMIP-II presents an intriguing avenue to probe the structural basis for the promiscuous receptor interaction. It is not clear whether common sites of vMIP-II have been optimized by the virus for multiple receptor interactions or distinctive binding determinants have been evolved for different receptors. This question was examined in the present study. A synthetic peptide approach was used to study the role of the N-terminus of vMIP-II in the recognition with two important chemokine receptors, CXCR4 and CCR5. The N-terminal region is most diverse among vMIP-II and other chemokines and, on the basis of the importance of N-termini in other chemokines (*9*), presumably critical for the unique function of vMIP-II. The V1 peptide corresponding to this region was shown to interact with CXCR4 and block its signal transduction and coreceptor function in mediating HIV-1 entry. This indicates that the N-terminus of vMIP-II is essential for its biological function through CXCR4. In contrast to its potent activities via CXCR4, V1 peptide did not display any interaction with CCR5 or inhibition of CCR5 signaling and coreceptor function. This is in contrast to native vMIP-II that can bind and block the function of both receptors. The lack of interaction of the N-terminal fragment of vMIP-II with CCR5 implies that other domains yet to be identified may mediate vMIP-II function via CCR5. We cannot rule out the alternative possibility that the peptide without other domains of the vMIP-II protein may fail to adopt conformations necessary for CCR5 recognition. However, this appears to be unlikely given the strong interaction of this peptide with another receptor CXCR4, suggesting that the peptide has proper structural elements for receptor binding. Taken together, results from this study have led us to propose that distinctive determinants in vMIP-II may mediate biological function via different receptors.

The important feature of the N-terminus of vMIP-II for CXCR4 recognition was further analyzed with truncated peptide analogues of V1. It has been suggested that a spatial cluster of positive residues in SDF-1 is critical for forming favorable electrostatic interaction with the negative charge surface of the extracellular domains of CXCR4 (*20*). A high positive charge is seen in several peptide and nonpeptide inhibitors of CXCR4, such as T22 (*21*), ALX40-4C (*22*), and AMD3100 (*23*). Interestingly, vMIP-II has a high net positive charge like SDF-1 despite the very low sequence homology between them. Since the V1 peptide derived from the N-terminus of vMIP-II also contains a number of positive charge residues, this raised the question whether these residues may play a role in receptor interaction. This hypothesis was tested with the V2 peptide that retains all positive residues in the core region of the V1 peptide. The

loss of activity in the V2 peptide seemed to argue against a primary role of the positive residues in receptor binding. Alternatively, the first five residues of vMIP-II may be more critical, and their removal in the V2 peptide likely explains the loss of activity. This seems to be consistent with observations made for other chemokines that the first several residues at the N-terminus are most important for biological function (*19*, *24*, *25*). The role of the first five residues of the N-terminus of vMIP-II was further demonstrated by V3, a shorten analogue containing only the N-terminal half of the V1 peptide, which retained some activity in CXCR4 binding (Figure 1).

The V1 peptide could be a promising lead for the development of high-affinity ligands for CXCR4. Even though a direct comparison with other chemokine-derived peptides cannot be made due to the difference in binding assay protocols, the relative affinity of the V1 peptide as compared with other CXCR4 binding peptides may be estimated by comparing these peptides with native SDF-1. In the current study, the V1 peptide was shown to compete with the CXCR4 binding of anti-CXCR4 mAb 12G5 and ^{125}I-SDF-1α with IC_{50} values of 640 and 190 nM, respectively (Table 2 and Figure 1), which are about 33- and 70-fold less potent than SDF-1, respectively. This compares favorably with other reported peptides derived from the N-terminus of SDF-1 which are about 82−1000-fold less potent than SDF-1 (*18*, *19*). In addition to its relatively high CXCR4 affinity among the chemokine-derived peptides reported so far, the V1 peptide possesses other interesting biological properties such as the induction of CXCR4 internalization (unpublished results). It was noted that the potency of the V1 peptide in the cell−cell fusion assay (Figure 2) was much lower than that in the competition binding assay (Figure 1 and Table 2). A similar discrepancy in potency between these two assays was also observed for SDF-1 which showed an IC_{50} of 2.7 nM in the ^{125}I-SDF-1α competitive binding assay (Figure 1 and Table 2) but had only 45% inhibition of cell−cell fusion even at 200 nM (Figure 2). These could be due to the relative insensitivity of the cell−cell fusion assay for the quantitative determination of potency of anti-HIV agents as previously reported by others (*17*). Therefore, the activity of the V1 peptide as well as the control SDF-1 could be underestimated in the cell−cell fusion assay. As compared with the cell−cell fusion assay, the inhibitory activity of the V1 peptide in the chemotaxis assay was much higher with an IC_{50} of about 1 μM, which was more consistent with its CXCR4 binding potency (Figure 4).

In summary, the characterization of precise binding sites within vMIP-II for CXCR4 and CCR5 is a critical step toward understanding the molecular mechanism of vMIP-II function and development of broad-spectrum HIV inhibitors. Here we report the identification of the N-terminus, particularly the first five residues, of vMIP-II as an important binding site for CXCR4. We have shown that a synthetic peptide derived from this region displays widely different interactions with CXCR4 and CCR5, thus providing experimental support for the notion that distinctive sites within vMIP-II may mediate interactions with different chemokine receptors. With its high CXCR4 receptor binding affinity and potent antagonistic effects, this vMIP-II-derived peptide could be a lead for the further development of novel small molecular agents that prevent the cellular entry of HIV via CXCR4 coreceptor.

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REFERENCES

1. Wells, T. N. C., Power, C. A., Lusti-Narasimhan, M., Hoogewert, A. J., Cooke, R. M., Chung, C., Peitsch, M. C., and Proudfoot, A. E. I. (1996) *J. Leukocyte Biol. 59*, 53−60.
2. Broder, C., and Berger, E. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 9004−9008.
3. Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., and Lusso, P. (1995) *Science 270*, 1811−1815.
4. Bleul, C. C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., and Sodroski, J. (1996) *Nature 382*, 829−833.
5. Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J. L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J. M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggiolini, M., and Moser, B. (1996) *Nature 382*, 833−835.
6. Moore, P. S., Boshoff, C., Weiss, R. A., and Chang, Y. (1996) *Science 274*, 1739−1744.
7. Boshoff, C., Endo, Y., Collins, P. D., Takeuchi, Y., Reeves, J. D., Schweickart, V. L., Siani, M. A., Sasaki, T., Williams, T. J., Gray, P. W., Moore, P. S., Chang, Y., and Weiss, R. A. (1997) *Science 278*, 290−294.
8. Kledal, T. N., Rosenkilde, M. M., Coulin, F., Simmons, G., Johnsen, A. H., Alouani, S., Power, C. A., Luttichau, H. R., Gerstoft, J., Clapham, P. R., Clark-Lewis, I., Wells, T. N. C., and Schwartz, T. W. (1997) *Science 277*, 1656−1659.
9. Clark-Lewis, I., Kim, K. S., Rajarathnam, K., Gong, J. H., Dewald, B., Moser, B., Baggiolini, M., and Sykes, B. D. (1995) *J. Leukocyte Biol. 57*, 703−711.
10. Endres, M. J., Clapham, P. R., Marsh, M., Ahuja, M., Turner, J. D., McKnight, A., Thomas, J. F., Stoebenau-Haggarty, B., Choe, S., Vance, P. J., Wells, T. N., Power, C. A., Sutterwala, S. S., Doms, R. W., Landau, N. R., and Hoxie, J. A. (1996) *Cell 87*, 745−756.
11. Alexander, W., Moss, B., and Fuerst, T. (1992) *J. Virol. 66*, 2934−2942.
12. Satoh, T., Aramini, J. M., Li, S., Friedman, T. M., Gao, J., Edling, A., Townsend, R., Germann, M. W., Korngold, R., and Huang, Z. (1997) *J. Biol. Chem. 272*, 12175−12180.
13. Li, S., Choksi, S., Shan, S., Gao, J., Korngold, R., and Huang, Z. (1998) *J. Biol. Chem. 273*, 16442−16445.
14. Zhou, N., Luo, Z., Hall, J. W., Luo, J., Han, X., and Huang, Z. (2000) *Eur. J. Immunol 30*, 164−173.
15. Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G., and Doms, R. W. (1996) *Cell 85*, 1149−1158.
16. Doranz, B. J., Lu, Z. H., Rucker, J., Zhang, T. Y., Sharron, M., Cen, Y. H., Wang, Z. X., Guo, H. H., Du, J. G., Accavitti, M. A., Doms, R. W., and Peiper, S. C. (1997) *J. Virol. 71*, 6305−6314.
17. Rucker, J., Doranz, B., Edinger, A., Long, D., Berson, J., and Doms, R. (1997) *Methods Enzymol. 288*, 118−133.
18. Loetscher, P., Gong, J. H., Dewald, B., Baggiolini, M., and Clark-Lewis, I. (1998) *J. Biol. Chem. 273*, 22279−22283.
19. Heveker, N., Montes, M., Germeroth, L., Amara, A., Trautmann, A., Alizon, M., and Schneider-Mergener, J. (1998) *Curr. Biol. 8*, 369−376.
20. Dealwis, C., Fernandez, E. J., Thompson, D. A., Simon, R. J., Siani, M. A., and Lolis, E. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 6941−6946.

21. Murakami, T., Nakajima, T., Koyanagi, Y., Tachibana, K., Fujii, N., Tamamura, H., Yoshida, N., Waki, M., Matsumoto, A., Yoshie, O., Kishimoto, T., Yamamoto, N., and Nagasawa, T. (1997) *J. Exp. Med. 186*, 1389−1393.
22. Doranz, B. J., Grovit-Ferbas, K., Sharron, M. P., Mao, S. H., Goetz, M. B., Daar, E. S., Doms, R. W., and O'Brien, W. A. (1997) *J. Exp. Med. 186*, 1395−1400.
23. Schols, D., Struyf, S., van Damme, J., Este, J. A., Henson, G., and De Clercq, E. (1997) *J. Exp. Med. 186*, 1383−1388.
24. Hèbert, C. A., Vitangcol, R. V., and Baker, J. B. (1991) *J. Biol. Chem. 266*, 18989−18994.
25. Crump, M. P., Gong, J. H., Loetscher, P., Rajarathnam, K., Amara, A., Arenzana-Seisdedos, F., Virelizier, J. L., Baggiolini, M., Sykes, B. D., and Clark-Lewis, I. (1997) *EMBO J. 16*, 6996−7007.

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