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# Activation of the Chemotactic Peptide Receptor FPRL1 in Monocytes Phosphorylates the Chemokine Receptor CCR5 and Attenuates Cell Responses to Selected Chemokines

Weiping Shen,* Paul Proost,† Baoqun Li,‡ Wanghua Gong,‡ Yingying Le,* Robert Sargeant,§ Philip M. Murphy,¶ Jo Van Damme,† and Ji Ming Wang*,[1]

**Laboratory of Molecular Immunoregulation, Division of Basic Sciences, National Cancer Institute, Frederick Cancer Research & Development Center, Building 560, Room 31-40, Frederick, Maryland 21702-1201; †Laboratory of Molecular Immunology, Rega Institute, University of Leuven, Leuven, Belgium; ‡Intramural Research Support Program, SAIC Frederick, National Cancer Institute, Frederick Cancer Research & Development Center, Frederick, Maryland 21702-1201; §Product Development, ProSci, Inc., 13100 Kirkham Way, Suite 207, Poway, California 92064; and ¶Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892*

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**FPRL1 is a seven-transmembrane (STM), G-protein coupled receptor which was originally identified as a low affinity receptor for the bacterial chemotactic formyl peptide and a high affinity receptor for the lipid metabolite lipoxin A4. We recently discovered that a number of peptides, including several synthetic domains of the HIV-1 envelope proteins and the serum amyloid A, use FPRL1 to induce migration and calcium mobilization in human monocytes and neutrophils. In this study, we report that a synthetic peptide domain of the V3 region of the HIV-1 envelope gp120, activates the FPRL1 receptor in monocytes and neutrophils. Furthermore, monocytes prestimulated with V3 peptide showed reduced response to several chemokines that use multiple cell receptors. This is associated with a rapid phosphorylation of the chemokine receptor CCR5 on the serine residues. Our study suggests that FPRL1, as a classical chemoattractant receptor, may play an important role in modulating monocyte activation in the presence of multiple stimuli.** 

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Abbreviations used: BM, binding medium; FPR, formyl peptide receptor; FPRL1, FPR-like 1; ETFR, epitope-tagged FPR; STM, seven-transmembrane.

[1] To whom correspondence should be addressed. Fax: 301-846-7042. E-mail: wangji@mail.ncifcrf.gov.

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Phagocytic leukocytes respond to a large number of chemoattractants with directional cell movement, activation of intergrins, generation of superoxide anions, and release of granule contents. These functions constitute the first-line of host defense against invading microorganisms. Numerous chemoattractants have been identified, which include the "classical" chemoattractants such as N-formyl-methionyl-leucyl-phenylalanine (fMLP), activated complement component 5 (C5a), leukotriene B4 (LTB4) and platelet-activating factor (PAF) (1–3), and a superfamily of newly defined chemokines (4–6). Both classical chemoattractants and chemokines activate G-protein coupled seven transmembrane (STM) receptors expressed not only on cells of hematopoietic origin, but also on other cell types. The interaction of these receptors with agonists plays a major role in the regulation of a number of pathophysiological processes.

FPRL1 is one of the classical STM receptors and was originally characterized as a low affinity receptor for the bacterial chemotactic peptide fMLP, which mobilizes $Ca^{2+}$ through this receptor at high concentrations (7–9). A lipid metabolite lipoxin A4 (LXA4) has been described as a high affinity agonist for FPRL1 by binding to and activating the GTPase through this receptor (10). We recently identified several synthetic peptide domains derived from the HIV-1 envelope proteins and a human acute phase protein serum amyloid A (SAA)

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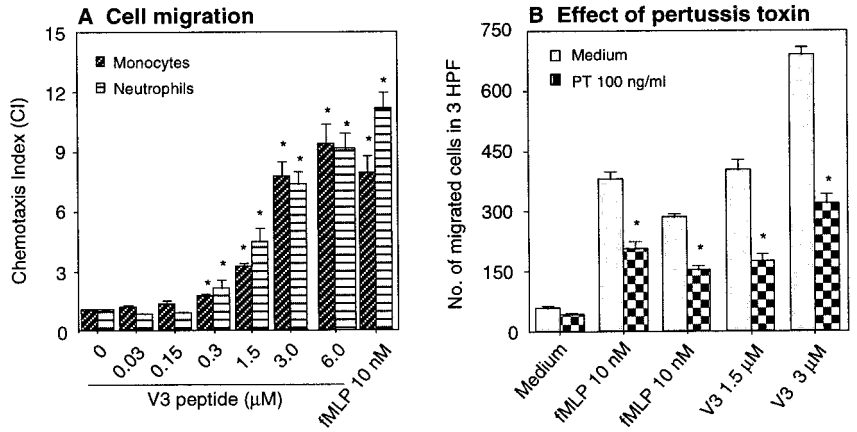


**FIG. 1.** Induction of phagocyte migration by synthetic V3 peptide. (**A**) Fold increase (chemotaxis index) of human phagocyte migration in response to V3 peptide over control medium. * $P < 0.01$, compared with spontaneous migration. (**B**) Inhibition of monocyte migration in response to V3 peptide by pertussis toxin (PT). Cells preincubated with 100 ng/ml pertussis toxin (PT) at 37°C for 30 min were washed and examined for migration induced by fMLP and V3 peptide. * $P < 0.01$, compared with migration of monocytes incubated with medium alone (Medium).

that induce migration and $Ca^{2+}$ mobilization in human phagocytic leukocytes by preferentially activating FPRL1 (11–13). These observations expanded the agonist spectrum of FPRL1 and suggested a greater capability of FPRL1 to host a variety of synthetic and endogenously produced agonists that do not bear significant sequence similarities. In this study, we report that a synthetic peptide domain derived from the V3 region of the HIV-1 gp120 is also a chemotactic agonist for FPRL1. Furthermore, activation of FPRL1 by V3 peptide resulted in attenuation of monocyte responses to selected chemokines in association with the serine phosphorylation of the chemokine receptor CCR5.

## MATERIALS AND METHODS

*Reagents and cells.* Synthetic V3 peptide (33 amino acid) of the HIV-1 gp120 (MN) (TRPNYNKRKRIHIGPGRAFYTTKNIIGTIR-QAH-$NH_2$) was kindly provided by the NIH AIDS Research and Reference Reagents Program (Bethesda, MD). This peptide was also synthesized on a preparative scale (0.25 mmol) by Fmoc chemistry on a 433A peptide synthesizer (PE Biosystem, Foster City, CA) (14) and purified by RP-HPLC on a Resource RPC column (Amersham Pharmacia Biotech, Uppsala, Sweden). The sequence of the synthetic peptide was confirmed by Edman degradation on a 477A/120A protein sequencer (PE Biosystems). Recombinant human chemokines and SAA were purchased from Pepro Tech Inc. (Rocky Hill, NJ). The chemotactic peptide fMLP and horse radish peroxidase conjugated goat anti-rabbit IgG were purchased from Sigma (St. Louis, MO). Protein A Sepharose CL-4B beads were purchased from Pharmacia Biotech (Piscataway, NJ). Polyclonal rabbit anti-phosphoserine antibody was from Zymed Laboratory Inc. (South San Francisco, CA). Polyclonal rabbit anti-CCR5 was from ProSci, Inc. (Poway, CA).

Human peripheral blood monocytes and neutrophils were separated from Buffy-Coats (NIH Clinical Center, Transfusion Medicine Department, Bethesda, MD) as described previously (12). Rat basophilic leukemia cells (RBL-2H3) transfected with an epitope-tagged cDNA coding for the high affinity fMLP receptor, FPR (designated ETFR cells), were a kind gift of Drs. H. Ali and R. Snyderman (Duke University, Durham, NC). Human embryonic kidney epithelial 293 cells transfected with cDNA encoding FPRL1 (FPRL1/293 cells) were established as previously described (7).

*Chemotaxis assays.* The migration of cells was assessed by a 48-well micro-chemotaxis chamber technique as described (13). Chemoattractants were placed in the wells of the lower compartment of the chemotaxis chamber. Cell suspension was placed in the wells of the upper compartment. Two compartments were separated by a polycarbonate filter (Neuroprobe, CabinJohn, MD, 5 $\mu$m pore-size for monocytes and neutrophils, 10 $\mu$m pore size for receptor transfectants). The filters used for migration of cell lines transfected with receptor genes were precoated with 50 $\mu$g/ml collagen type I (Collaborative Biomedical Products, Bedford, MA). The chamber was incubated at 37°C in humidified air with 5% $CO_2$. After incubation (1 h for neutrophils, 1.5 h for monocytes, and 5 h for receptor transfected cell lines), the filter was removed, fixed and stained with LeukoStat (Fisher Scientific, Pittsburg, PA). The migrated cells in three high-powered fields (400×) were counted by light microscopy after coding the samples. The results are expressed as the mean (± SE) value of the migration in triplicate samples and are representatives of at least three experiments performed. Cell migration was also presented as chemotaxis index (CI) representing the fold increase in cell migration induced by stimulants over control medium.

*Calcium mobilization.* Calcium mobilization was measured by incubating $2 \times 10^7$/ml cells in loading medium (DMEM, 10% FBS) with 5 $\mu$M Fura-2 AM (Molecular Probes, Eugene, OR) for 30 min at room temperature in the dark. The dye-loaded cells were washed 3 times with loading medium and resuspended in saline buffer [138 mM NaCl, 6 mM KCl, 1 mM $CaCl_2$, 10 mM HEPES (pH 7.4), 5 mM Glucose, 0.1% BSA] at a concentration of $1 \times 10^6$/ml. The cells were then transferred into quartz cuvettes (2 ml) which were placed in a luminescence spectrometer (LS-50B, Perkin-Elmer, Beaconsfield, England). Stimulants at different concentrations were added in a volume of 20 $\mu$l to each cuvette at the indicated time points. The ratio of fluorescence at 340 and 380 nm wavelength was calculated using a FL WinLab program (Perkin-Elmer).

*Phosphorylation of CCR5.* Monocytes stimulated with different concentrations of stimulants for indicated time periods at 37°C were lysed for 20 min on ice with periodic mixing in lysis buffer [1% Triton X-100, 20 mM Tris–HCl (pH 8.0), 137 mM NaCl, 15% glycerol, 5 mM EDTA] containing phosphatase and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 5 $\mu$g/ml aprotinin, 5 $\mu$g/ml leupeptin, 1

mM sodium orthovanadate, 1 mM EGTA). Cell lysates were precleaned with 30 $\mu$l of washed Protein A Sepharose beads (15 $\mu$l packed beads) at 4°C for 1 h and 1 $\mu$g of polyclonal anti-phosphoserine antibody was added to 200 $\mu$g cell lysates diluted with 2× immunoprecipitation (IP) buffer [1×: 1% Triton X-100, 10 mM Tris–HCl (pH 7.4), 137 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium, 0.2 mM PMSF, 0.5% NP-40]. The reaction mixture was incubated at 4°C overnight under constant rocking. The immunecomplex was captured by adding 50 $\mu$l of washed Protein A sepharose beads (25 $\mu$l packed beads) and incubating the reaction mixture at 4°C for an additional 2 h. The beads were spun down (10 s at 14,000 rpm), drained off the supernatants, washed 3 times with ice-cold 1× IP buffer, then were resuspended in 30 $\mu$l 2× Laemmli sample buffer (126 mM Tris–HCl, 20% Glycerol, 4% SDS, 0.005% Bromophenol Blue. Novex, San Diego, CA) and boiled for 5 min to elute the immune complex. After electrophoresis on 10% SDS–PAGE precast gel (Novex, San Diego, CA), the proteins were transferred to Immobilon P membranes (Millipore Corp., Bedford, MA). The membrane was blocked in freshly prepared PBS containing 3% dry milk at 4°C for 2 h, then was incubated with 1 $\mu$g/ml of polyclonal anti-CCR5 antibody overnight at 4°C followed by washing 3 times with PBS-T (0.05% Tween 20). The membrane was incubated with a horse radish peroxidase-conjugated goat anti-rabbit IgG at 1:5000 dilution in PBS containing 3% dry milk for 1 h at room temperature with agitation. After washing 3 times with PBS-T, the membrane was incubated with SuperSignal Chemiluminescent Substrate Stable Peroxide Solution (PIERCE, Rockford, IL) for 1 min, and exposed to BIOMAX-MR film (Eastman Kodak Company, Rochester, NY).

*Statistical analysis.* All experiments were performed at least three times and the results presented are from representative experiments. The significance of the difference between test and control groups was analyzed using the Student's *t*-test. A chemotaxis index (CI) of 1.8 or greater is statistically significant.

## RESULTS

Since V3 region of the HIV-1 gp120 is thought to play a major role in determining the cell tropism of the HIV-1 (15–18), synthetic V3 peptides have been used to examine whether they interact directly with the viral fusion co-factors such as CCR5 and CXCR4 which in the presence of CD4 mediate the fusion of monocyte or T lymphocyte tropic HIV-1 respectively (19). Synthetic V3 domains either linear or cyclized of the T-tropic HIV strains IIIB and EL1, have been reported to directly bind CXCR4 and induce migration of both $CD4^+$ and $CD8^+$ T lymphocytes (19). We also tested a synthetic V3 peptide domain derived from the MN strain of the HIV-1 and found that this V3 peptide is a potent chemoattractant for human monocytes and neutrophils (**Fig. 1A**) but only weakly for T lymphocytes (data not shown). Since the chemotaxis response of human phagocytes to V3 peptide was inhibited by preincubation of the cells with pertussis toxin (**Fig. 1B**), we postulated that this V3 peptide activated G protein coupled STM receptor(s).

In an effort to characterize the nature of the receptor(s) used by this V3 peptide in phagocytes, we found that both monocytes and neutrophils mobilized $Ca^{2+}$ after stimulation with the peptide (**Figs. 2A** and **2B**). V3 peptide did not attenuate the cell response to a number of chemokines including SDF-1$\alpha$ which is a cognate ligand for the receptor CXCR4 (**Figs. 2C–2F**). In contrast, the signaling of V3 peptide in phagocytes was attenuated by high concentration of fMLP or vice versa (**Figs. 2G** and **2H**), suggesting V3 peptide might activate a low affinity receptor for fMLP. This hypothesis was confirmed by experiments using cells trans-

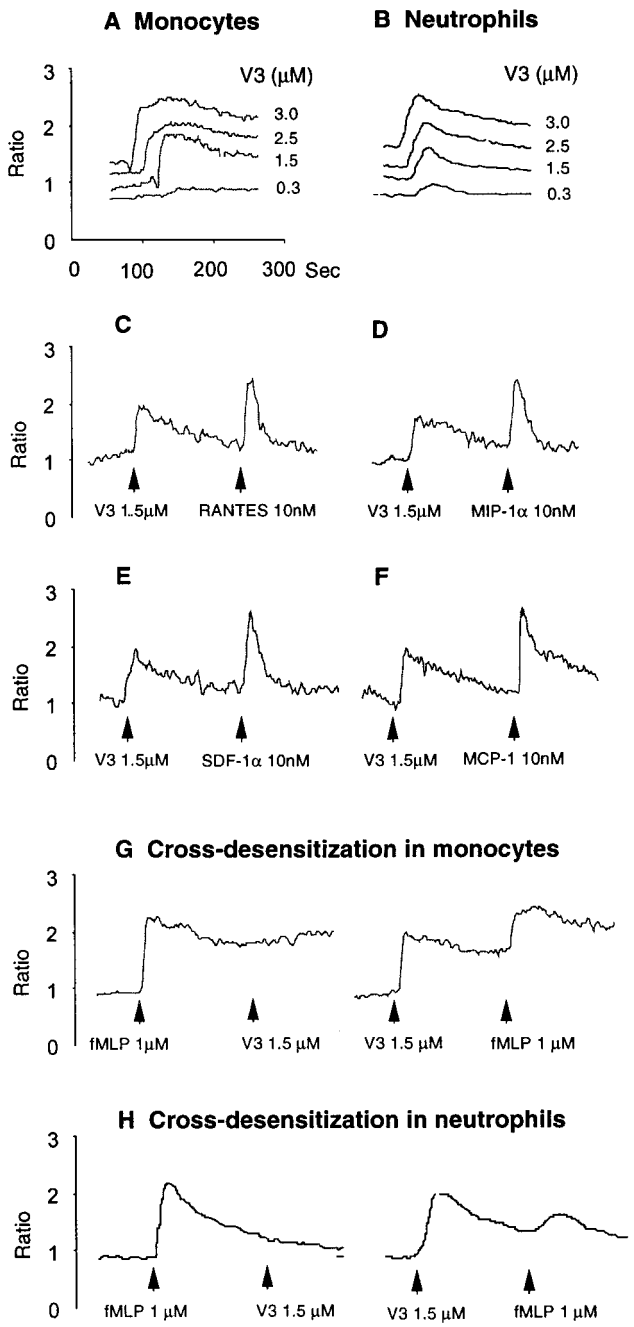


**FIG. 2.** Calcium mobilization in phagocytes induced by V3 peptide and cross desensitization with fMLP. (**A** and **B**) $Ca^{2+}$ mobilization ing monocytes and neutrophils induced by V3 peptide. (**C–F**) Effect of V3 peptide (1.5 $\mu$M) on chemokine induced $Ca^{2+}$ flux in monocytes. (**G** and **H**) Cross-desensitization between V3 peptide (1.5 $\mu$M) and fMLP (1 $\mu$M) for signaling in monocytes and neutrophils.

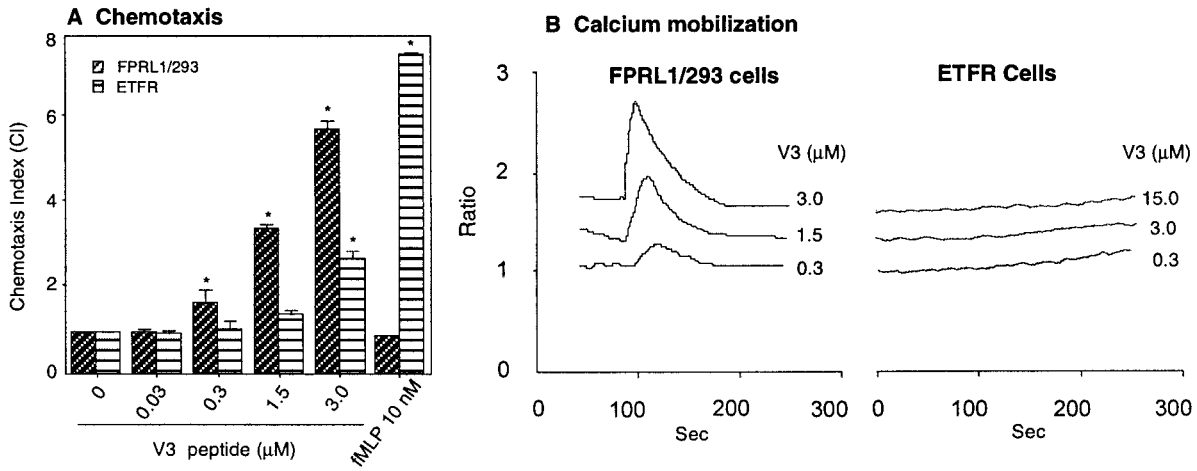


**FIG. 3.** Migration and $Ca^{2+}$ flux in FPR (ETFR) and FPRL1 (FPRL1/293) expressing cells induced by V3 peptide. (**A**) Migration of the receptor expressing cells in response to V3 peptide. fMLP was used as a control. (**B**) $Ca^{2+}$ flux induced by V3 peptide in FPRL1/293 ETFR cells.

fected to express FPRL1, a STM receptor known to interact with fMLP with low affinity (7, 9). As predicted, V3 peptide induced both $Ca^{2+}$ mobilization and chemotaxis of HEK293 cells transfected to express FPRL1 (**Figs. 3A** and **3B**). In contrast, V3 peptide induced a low level of chemotaxis response (**Fig. 3A**), but not $Ca^{2+}$ mobilization in cells overexpressing FPR (**Fig. 3C**), the high affinity fMLP receptor (ETFR cells). These results showed that FPRL1 is a major functional receptor used by V3 peptide.

We have shown that stimulation of human phagocytes with V3 peptide did not attenuate the $Ca^{2+}$ flux response to subsequent challenge with chemokines, indicating the V3 peptide does not share receptors with the chemokines. However, since the V3 peptide preferentially activated a STM receptor FPRL1, we next tested whether a prolonged treatment of monocytes with V3 peptide could attenuate the cell response to chemokines, presumably based on "heterologous" desensitization of the receptors, a process that has been documented for a number of STM chemoattractant receptors and may represent an important regulatory element in cell response in the presence of multiple stimulants. Chemokines RANTES, SDF-1$\alpha$, and MCP-1 all induced a significant $Ca^{2+}$ mobilization in monocytes preincubated with the culture medium (**Fig. 4**). However, when monocytes were preincubated with V3 peptide (1.5 $\mu$M) at 37°C for 60 min, the cell response to RANTES and SDF-1$\alpha$, was significantly reduced (**Figs. 4A** and **4B**). In contrast, monocytes preincubated with the protein kinase C (PKC) inhibitor staurosporine followed by V3 peptide showed almost normal $Ca^{2+}$ mobilization in response to RANTES or SDF-1$\alpha$. These results suggest that V3 peptide, by activating FPRL1 in monocytes, caused a heterologous desensitization of the cell response to these chemokines via a PKC mediated signal transduction pathway. On the other hand, the cell response to another chemokine, MCP-1, and the chemotactic peptide fMLP was only minimally affected by preincubation with V3 peptide (**Figs. 4C** and **4D**), suggesting that the susceptibility of the receptors to heterologous desensitization varies widely.

Since in heterologous desensitization of G-protein coupled STM receptors, the second messenger induced phosphorylation of the receptor plays an important role (20), we investigated whether the CC chemokine receptor CCR5 could be phosphorylated by stimulation of monocytes with V3 peptide. In our preliminary experiments, CCR5 in both monocytes and receptor transfected HEK293 cells (CCR5/293 cells) was detected by immunoblotting with a polyclonal anti-CCR5 antibody as a dimerized protein species at about 75 kDa under non-reducing conditions, and an approximately 40 kDa species under reducing conditions (data not shown), in agreement with the results obtained by other investigators with CCR5 transfected cell lines (21). In addition, stimulation of CCR5/293 cells, but not monocytes, with specific chemokines further promoted the dimerization of CCR5 which could be detected even under non-reducing conditions (data not shown). Since CCR5 has been reported to undergo phosphorylation on the serine residues at its carboxyl terminus (22, 23), we used an anti-phosphoserine antibody to immunoprecipitate cell lysates followed by immunoblotting with the polyclonal anti-CCR5 antibody to detect the phosphorylation of CCR5. We observed that CCR5 could be rapidly phosphorylated by stimulation of the cells with CCR5 chemokine agonists MIP-1$\alpha$, RANTES, and MIP-1$\beta$ (**Figs. 5A, 5B** and data not shown) with the maximal level of phosphorylation occurring at 1 min. Immunoblotting of the total monocyte lysates with anti-CCR5 antibody yielded a protein species at the same molecular weight position (**Fig. 5C**). Although

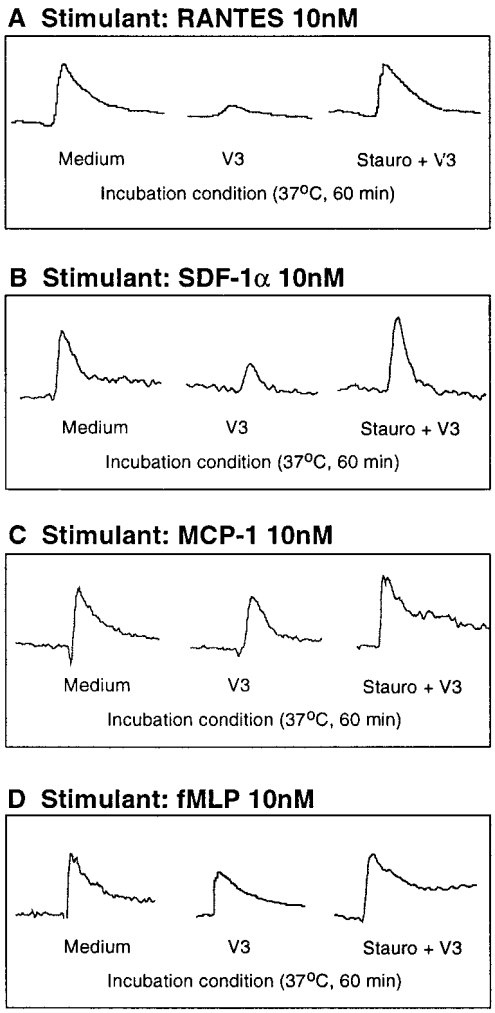


**FIG. 4.** Effect of preincubation with V3 peptide on monocyte $Ca^{2+}$ mobilization in response to chemokines. Monocytes loaded with Fura-2 were preincubated with medium (Medium) or V3 peptide (V3) for 60 min at 37°C. After washing, the cells were measured for $Ca^{2+}$ mobilization in response to chemokines (10 nM) RANTES (**A**), SDF-1$\alpha$ (**B**), MCP-1 (**C**) or fMLP (10 nM) (**D**). In parallel experiments, cells were first treated with staurosporine (2.5 ng/ml, 30 min at 37°C) followed by V3 peptide (1.5 $\mu$M, 37°C, 60 min, Stauro + V3), then were measured for $Ca^{2+}$ flux in response to chemokines or fMLP.

stimulation of monocytes with V3 peptide also induced significant serine phosphorylation of CCR5, the maximal level of CCR5 phosphorylation was observed at 15 min after stimulation (**Figs. 5D** and **5E**). These results are in agreement with the notion that heterologous receptor desensitization may require a longer incubation time with the stimulants and an accumulation of second messengers that culminates in phosphorylation of an unrelated STM receptor. In fact, when monocytes were pretreated with the PKC inhibitor staurosporine, V3 peptide no longer significantly induced phosphorylation of CCR5, whereas staurosporine treatment did not affect MIP-1$\beta$-induced CCR5 phosphorylation (**Fig. 5F**). Thus, the mechanism of agonist-induced CCR5 phosphorylation differs from that induced by the FPRL1 ligand V3 peptide. To confirm the effect of FPRL1 activation on CCR5 phosphorylation, we used SAA, a known chemotactic agonist of FPRL1 (13). As shown in **Figs. 5G** and **5H,** treatment of monocytes with SAA indeed induced a rapid and dose-dependent serine phosphorylation of CCR5. The bacterial chemotactic peptide fMLP which uses primarily the receptor FPR in monocytes, also induced CCR5 phosphorylation (**Fig. 5E**) with a maximal level of effect at 60 min (data not shown). These results indicate that CCR5 is subjected to phosphorylation induced by activation of either FPR or FPRL1 in monocytes.

## DISCUSSION

In this study, we showed that a synthetic V3 peptide domain of gp120 from HIV-1 MN strain induced migration and $Ca^{2+}$ mobilization in human monocytes and neutrophils by preferentially activating a G protein-coupled STM receptor FPRL1. We also observed that activation of monocytes induced phosphorylation of the chemokine receptor CCR5 in association with attenuation of the cell signaling in response to selected chemokines. This is an unexpected finding in that the V3 domain of the HIV-1 gp120 is thought to play an important role in determining the tropism of the virus (15–18). Although the synthetic domain from M-tropic HIV-1 has not been documented to directly interact with the fusion co-receptor CCR5, the synthetic and cyclized V3 peptide domain of the T-tropic HIV-1 strains (IIIB and ELI) has been reported to directly bind to the fusion co-receptor CXCR4 (19). In our study, a cyclized V3 peptide derived from HIV-1 gp120 of the MN strain (NIH AIDS Research and Reference Reagent Program) did not activate monocytes or neutrophils and both linealized or cyclized V3 peptide of the gp120 (MN) did not interact with CXCR4 (data not shown). Therefore, a secondary or tertiary structure of the peptide may be crucial for the formation of a specific binding domain for CXCR4 and V3 domains of different HIV-1 strains may have divergent capacity to interact directly with chemokine fusion coreceptors. More research is required to address whether the ability of the V3 peptide domain to directly interact with chemokine co-receptors is an intrinsic property for gp120 of all HIV-1 strains or if it is restricted to selected strains. By analogy, the interaction of linealized V3 peptide with FPRL1 as seen in our study also needs to be further investigated with domains derived from other HIV-1 strains.

FPRL1 was identified and molecularly cloned from a genomic DNA library of human phagocytic cells by low stringency hybridization of the cDNA library with the sequence of the prototype receptor FPR (7, 24–26). FPRL1 possesses 69% identity at the amino acid level

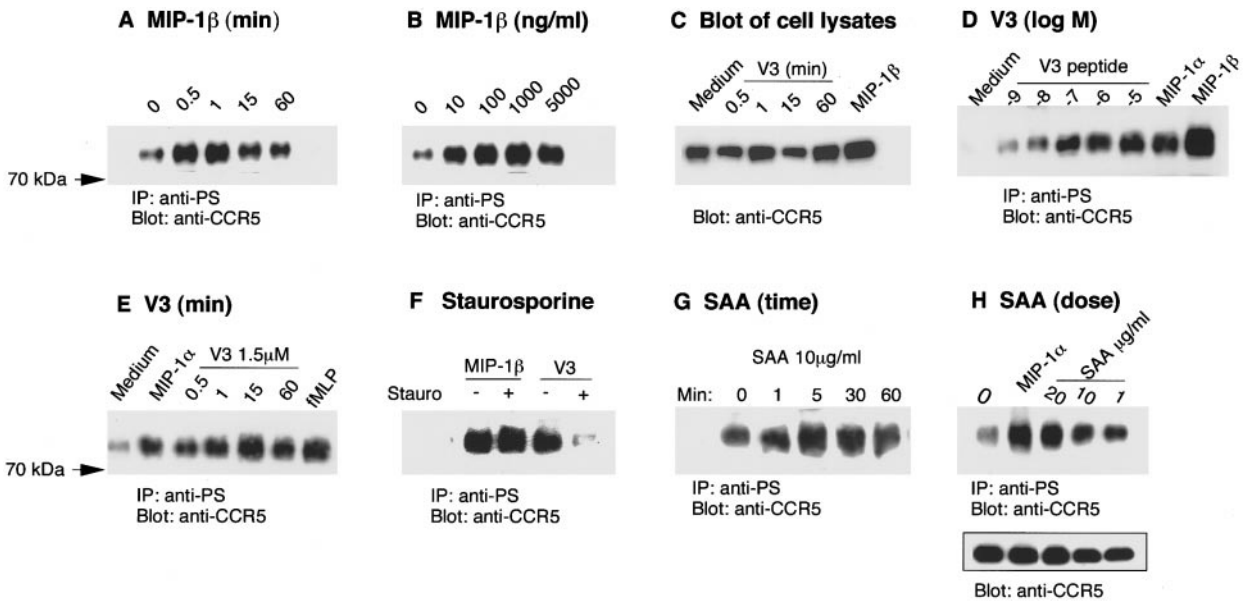


**FIG. 5.** Phosphorylation of CCR5 in monocytes induced by chemokines and FPRL1 agonists. (**A**) Monocytes treated with MIP-1$\beta$ at 100 nM for different time periods (min) at 37°C. (**B**) Monocytes treated with different concentrations of MIP-1$\beta$ for 1 min at 37°C. (**C**) Total lysates of monocytes treated with V3 peptide or MIP-1$\beta$ (1 min) were immunoblotted with anti-CCR5 antibody. (**D**) Monocytes treated with different concentration of V3 peptide for 60 min at 37°C, cells treated with MIP-1$\alpha$ (100 nM) and MIP-1$\beta$ (100 nM) for 1 min were used as control. (**E**) Monocytes treated with V3 peptide (1.5 $\mu$M) for different time points, cells treated with MIP-1$\alpha$ (100 nM) for 1 min or with fMLP (1 $\mu$M) for 60 min were used as control. (**F**) Effect of staurosporine (Stauro, 2.5 ng/ml, 30 min) on CCR5 phosphorylation induced by MIP-1$\beta$ (100 nM, 1 min at 37°C) or V3 peptide (1.5 $\mu$M, 60 min). (**G**) Monocytes treated with SAA at 10 $\mu$g/ml for different time periods (min) at 37°C. (**H**) Monocytes treated with different concentrations of SAA for 60 min at 37°C. Inset, Immunoblotting of 20 $\mu$g whole monocyte lysates with anti-CCR5 antibody.

to the prototype chemotactic peptide receptor FPR (7–9, 24–26), and both receptors are expressed by monocytes and neutrophils (26, 27). While fMLP is a high affinity agonist for FPR, it interacts with and induces $Ca^{2+}$ flux through FPRL1 only at high concentrations (7–9, 24–26). In our study, fMLP did not induce significant migration of FPRL1/293 cells at a concentration as high as 50 $\mu$M (13), suggesting that fMLP is not a full agonist for FPRL1. In contrast, V3 peptide induced both $Ca^{2+}$ flux and migration through FPRL1. Although FPRL1 was originally found to be expressed in monocytes and neutrophils, cells other than phagocytes have been shown to express FPRL1 (8, 28). Therefore, FPRL1 may play an important role in inflammatory and immunological responses in human cells. We recently identified several chemotactic agonists for FPRL1 including some synthetic peptide domains of the HIV-1 envelope protein (11, 12) and serum amyloid A (SAA) (13). In addition, several FPRL1 agonists have been isolated from a random peptide library (29). Furthermore, a lipid metabolite lipoxin A4 (LXA4) has been reported to be a high affinity agonist for FPRL1 (thus also termed LXA4R) (10). LXA4 is an eicosanoid generated in a number of inflammatory and immunological processes (30) yet appears to have opposing effects on proinflammtory responses of neutrophils versus monocytes (31–33). Thus differential activation of second messengers induced by LXA4 in different cell types was postulated. It should be noted that the FPRL1 agonists identified thus far do not bear substantial sequence homology and further study is required to define the structural basis for the promiscuous property of FPRL1.

Since FPRL1 is highly homologous to the prototype FPR and both receptors are sensitive to pertussis toxin, they may share common features of second messenger activation. The binding of FPR by agonists results in a G protein-mediated signaling cascade leading to cell adhesion, chemotaxis, release of oxygen intermediates, enhanced phagocytosis, and bacterial killing, as well as MAP kinase activation and gene transcription (8, 9). Activation by fMLP may also result in heterologous desensitization of the subsequent cell response to other G-protein receptor ligands (20), including chemokines. This is supported by our observation that fMLP at a concentration preferentially activating FPR caused phosphorylation of CCR5 in monocytes. In our previous study, incubation of human phagocytes with FPRL1 agonist SAA resulted in a reduction of cell response to a number of chemoattractants (34), suggesting that activation of FPRL1 may also activate signaling events that cause desensitization of other G-protein coupled chemotactic receptors. In support of this hypothesis, we recently found that a peptide domain derived from

HIV-1 gp120, preferentially activated phagocytes through FPRL1 and down regulated the expression and function of two chemokine receptors CCR5 and CXCR4 (12) which act as key fusion cofactors for HIV-1. We further demonstrate that incubation of monocytes with V3 peptide or SAA resulted in serine phosphorylation of CCR5 accompanied by attenuation of cell signaling in response to selected chemokine ligands. Since receptor heterologous desensitization was considered an important pathophysiological process in concerted immune responses, FPRL1 may play an important role in regulating monocyte response to multiple chemotactic stimulants. Therefore, immuneregulatory agents could be designed based on the interaction of FPRL1 and its agonist.

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