Interleukin-8-mediated Heterologous Receptor Internalization Provides Resistance to HIV-1 Infectivity

ROLE OF SIGNAL STRENGTH AND RECEPTOR DESENSITIZATION*

Received for publication, November 18, 2002, and in revised form, February 7, 2003
Published, JBC Papers in Press, February 19, 2003, DOI 10.1074/jbc.M211745200

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Human immunodeficiency virus type 1 (HIV-1) entry into CD4+ cells requires the chemokine receptors CCR5 or CXCR4 as co-fusion receptors. We have previously demonstrated that chemokine receptors are capable of cross-regulating the functions of each other and, thus, affecting cellular responsiveness at the site of infection. To investigate the effects of chemokine receptor cross-regulation in HIV-1 infection, monocytes and MAGIC5 and rat basophilic leukemia (RBL-2H3) cell lines co-expressing the interleukin-8 (IL-8 or CXCL8) receptor CXCR1 and either CCR5 (ACCR5) or CXCR4 (ACXCR4) were generated. IL-8 activation of CXCR1, but not the IL-8 receptor CXCR2, cross-phosphorylated CCR5 and CXCR4 and cross-desensitized their responsiveness to RANTES (regulated on activation normal T cell expressed and secreted) (CCL5) and stromal derived factor (SDF-1 or CXCL12), respectively. CXCR1 activation internalized CCR5 but not CXCR4 despite cross-phosphorylation of both. IL-8 pretreatment also inhibited CCR5- but not CXCR4-mediated virus entry into MAGIC5 cells. A tail-deleted mutant of CXCR1, ΔCXCR1, produced greater signals upon activation (Ca2+ mobilization and phosphoinositide hydrolysis) and cross-internalized CXCR4, inhibiting HIV-1 entry. The protein kinase C inhibitor staurosporine prevented phosphorylation and internalization of the receptors by CXCR1 activation. Taken together, these results indicate that chemokine receptor-mediated HIV-1 cell infection is blocked by receptor internalization but not desensitization alone. Thus, activation of chemokine receptors unrelated to CCR5 and CXCR4 may play a cross-regulatory role in the infection and propagation of HIV-1. Since ΔCXCR1, but not CXCR1, cross-internalized and cross-inhibited HIV-1 infection to CXCR4, the data indicate the importance of the signal strength of a receptor and, as a consequence, protein kinase C activation in the suppression of HIV-1 infection by cross-receptor-mediated internalization.

Chemokines are a diverse gene family of chemotactic cytokines that induce leukocyte accumulation and activation at sites of inflammation (1–3). They also mediate tumor cell trafficking and metastasis and participate in many acute and chronic inflammatory diseases (4, 5). Chemokine functions are mediated via cell surface G-protein-coupled receptors that couple predominantly to Gαi (1–3, 18, 35). Chemokine receptors, most notably CCR5 and CXCR4, also serve as co-receptors for human immunodeficiency virus type 1 (HIV-1)1 entry into CD4+ cells (6, 7). To date, the relationship between the activation of these receptors and their role in HIV-1 infection is not well understood.

Like many members of the G-protein-coupled receptor family, CCR5 and CXCR4 become desensitized upon agonist exposure, resulting in a loss of cellular responsiveness to agonist followed by a decrease in the number of cell surface receptors (8–13). Phosphorylations of the carboxyl terminus of the receptors are responsible for the desensitization and down-regulation (8–13). We have previously shown that chemokine receptors cross-regulate the functions of each other (14, 35). The interleukin-8 (IL-8 or CXCL8) receptor CXCR1 cross-phosphorylated and cross-desensitized CCR1-mediated cellular responses to RANTES (CCL5) (14). The formyl peptide chemotractant receptor also cross-desensitized CCR5-mediated cellular responses to RANTES in monocytes and diminished the ability of RANTES to mediate HIV-1 entry and infection (15, 16).

While HIV-1 infection requires the CD4 receptor, the role of a chemokine receptor as the fusion cofactor depends on the target cell (17). Both macrophages and T lymphocytes express CCR5 and CXCR4 (18). Macrophages, however, utilize CCR5 for HIV-1 entry (M-tropism), whereas CD4+ T lymphocytes use CXCR4 (T-tropism) (18). In addition to CCR5 and CXCR4, macrophages and CD4+ T lymphocytes express other chemokine receptors including the IL-8 receptors CXCR1 and CXCR2 (1–8 × 106 receptors/cell) (1, 18–24). In the present study we sought to determine the role of cross-regulation by IL-8 receptors in CCR5- and CXCR4-mediated cellular activation and HIV-1 infection. For this purpose, monocytes and MAGIC5 and RBL-2H3 cells stably expressing different combination of receptors were used to study the mechanisms of cross-regulation among IL-8, CCR5, and CXCR4. The results demonstrate that IL-8 led to the cross-phosphorylation and cross-desensitization of both CCR5 and CXCR4. However, IL-8 down-regulated and

*This work was supported by National Institutes of Health Grants AI-38910 (to R. M. R.) and DE-03738 (to R. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: HIV-1, human immunodeficiency virus type 1; IL-8, interleukin-8; CXCR1, IL-8 receptor A; CXCR2, IL-8 receptor B; FMA, phorbol 12-myristate 13-acetate; RANTES, regulated on activation normal T cell expressed and secreted; SDF, stromal derived factor; RBL, rat basophilic leukemia; MGSA, melanoma growth-stimulating activity; HA, hemagglutinin; PKC, protein kinase C.
inhibited HIV-1 infection to CCR5 but not CXC RX4. Since CCR5 is a target for the entry of primary viruses in monocytes these results suggest a selective role for IL-8 in limiting HIV-1 infection through this receptor.

**EXPERIMENTAL PROCEDURES**

**MATERIALS**—(32P)Orthophosphate (8500–9120 Ci/mmol), IL-8, and 125I-RANTES were purchased from PerkinElmer Life Sciences. IL-8 (monocyte-derived), melanoma growth-stimulating activity (MGSA) or CXC(1), RANTES, MIP-1α (CCL4), and SDF-1 were purchased from PeproTech. Genetin (G418) and all tissue culture reagents were purchased from Invitrogen. Monoclonal 12CA5 antibody, protein G-agarose, and protease inhibitors were purchased from Roche Applied Science. Anti-human IL-8RA (CXCR1) and IL-8RB (CXCR2) antibodies were purchased from Pharmingen. Indo-1 acetoxymethyl ester and pluronic acid were purchased from Molecular Probes. Powerblock 12-myristate 13-acetate (PMA) and M2-FLAG antibody were purchased from Sigma. FuGENE 6 was purchased from Roche Applied Science. The enzyme-linked immunosorbent assay was obtained from PerkinElmer Life Sciences. All other reagents are from commercial sources.

**Isolation of Monocytes**—Monocytes were isolated from heparinized human blood on a multiple density gradient and enriched for mononuclear cells as described previously (25, 26).

**Construction of Epitope-tagged CXCR1, CXCR4, and CCR5**—Nucleotides encoding the nine-amino acid (YPYDVPDYA) hemagglutinin (HA) (CXCR1 and ΔCXCR1) or the octapeptide (DYKDDDDK) FLAG (CCR5 and CXCR4) epitope sequences were inserted between the amino-terminal initiator methionine and the second amino acid of each cDNA by polymerase chain reaction as described previously (9, 10, 27). The resulting PCR products were cloned into the eukaryotic expression vector pCMV-3, and the receptors were sequenced to confirm the intended mutations and lack of secondary mutations.

**Cell Culture and Transfection**—RBL-2H3 cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 15% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 mg/ml) (27). RBL-2H3 cells (1 × 107 cells) were transfected by electroporation with 50 μg of pCMV-3 containing the receptor cDNAs, and geneticin-resistant cells were selected by G418. pCMV-3 containing 10 μg/ml bovine serum albumin and washed three times with phosphate-buffered saline and cultured in fresh medium for 90 min. Then labeled cells were stimulated with the indicated ligands for an additional 24 h. CXCR1 and ΔCXCR1 expression was monitored by fluorescence-activated cell sorter analysis. Levels of protein expression were monitored by fluorescence-activated cell sorter analysis and Western blotting using 12CA5 (HA)- and M2 (FLAG)-specific antibodies.

**Radioligand Binding Assays and Receptor Internalization**—RBL-2H3 cells were subcultured overnight in 24-well plates (0.5 × 106 cells/well) in growth medium. Cells were then rinsed with Dulbecco's modified Eagle's medium supplemented with 15% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 mg/ml) (27). RBL-2H3 cells (1 × 107 cells) were transfected by electroporation with 50 μg of pCMV-3 containing the receptor cDNAs, and geneticin-resistant cells were cloned into a single cell by fluorescence-activated cell sorter analysis. Levels of protein expression were monitored by fluorescence-activated cell sorter analysis and Western blotting using 12CA5 (HA)- and M2 (FLAG)-specific antibodies.

**GTPase Activity**—Cells were treated with appropriate concentrations of stimulants, and membranes were prepared as described previously (9, 14). GTPase activity using 10–20 μg of membrane preparations were carried out as described previously (14, 27).

**Phosphoinositide Hydrolysis and Calcium Measurement**—RBL-2H3 cells were subcultured overnight in 96-well culture plates (50,000 cells/well) in isositol-free medium supplemented with 10% dialyzed fetal bovine serum and 1 μCi/ml [3H]inositol. The generation of inositol 1,4,5-trisphosphate and [3H]inositol was determined with a MicroLumatPlus LB96V microplate luminometer.

**RESULTS**

**Cross-desensitization of CCR5- and CXCR4-mediated Ca2+ Mobilization in Human Monocytes**—To study the cross-desensitization of CCR5 and CXCR4, intracellular Ca2+ mobilization in monocytes was elicited by RANTES and SDF-1 and used as a measure of CCR5 and CXCR4 activation, respectively. As shown in Fig. 1A, IL-8, which activates both CXCR1 and CXCR2, cross-desensitized Ca2+ responses to RANTES and SDF-1, MGSA, which only activates CXCR2, had no effect on RANTES or SDF-1. RANTES and SDF-1 pretreatment attenuated responses to both MGSA and IL-8 (Fig. 1A). RANTES, SDF-1, IL-8, and MGSA homologously desensitized (∼80%) responses to a second dose of the same ligand (Fig. 1B).

**Cross-inhibition of CCR5- and CXCR4-mediated HIV-1 Infection by IL-8**—To analyze whether IL-8 inhibited entry to CCR5 and CXCR4, MAGC5 cells were infected with a lentiviral CXCR1 expression vector virus. Cells were pretreated with or without IL-8 (100 nM) and infected with NL-Luc-ADA (R5-tropic) or NL-Luc-HXB (X4-tropic), and luciferase activity was measured. As shown in Fig. 2, IL-8 pretreatment inhibited by ∼50% CCR5-mediated R5-tropic virus infection but had no effect in X4-tropic infection to CXCR4.

**Co-expression, Characterization, and Cross-desensitization of CXCR1, CCR5, and CXCR4 in RBL-2H3 Cells**—To study the mechanism of cross-regulation among CXCR1, CCR5, and CXCR4, single transfected RBL cells expressing FLAG-tagged CCR5 (CCR5-RBL) or CXCR4 (CXCR4-RBL) were first generated and characterized. The Kd values for RANTES binding to 2 Richardson, R. M., Marjoram, R. J., Barak, L. S., and Snyderman, R. (2003) J. Immunol. 70, 2904–2911.
the CCR5 (4.3 ± 0.5 nM) and for SDF-1 binding to CXCR4 (5.7 ± 1 nM) were similar to those previously reported (9, 10). CCR5 induced a comparable peak of intracellular Ca2+ mobilization in response to both RANTES (Fig. 3A) and MIP-1β (data not shown) but not IL-8, MGSA, or SDF-1 (Fig. 3A). CXCR4 was also specific for SDF-1-mediated Ca2+ mobilization (Fig. 3B). Cells expressing CXCR1 were specific for IL-8 (data not shown).

In cells co-expressing CXCR1 and CCR5 (ACCR5-RBL) IL-8 pretreatment cross-desensitized intracellular Ca2+ mobilization to both RANTES (58%) and MIP-1β (56%) (Table I). IL-8 also inhibited SDF-1 (35%) mediated Ca2+ response in cells co-expressing CXCR1 and CXCR4 (ACXCR4-RBL). Inhibition of responses to CCR5 was greater than that of CXCR4 (56–58 versus 35%, respectively). IL-8-mediated Ca2+ mobilization was also attenuated by pretreatment of the cells with RANTES, MIP-1β, or SDF-1 (Table I). IL-8, RANTES, MIP-1β, and SDF-1 also homologously desensitized (~90%) the response to a second dose of the same ligand (Table I). Pretreatment of ACCR5 (Fig. 4A) or ACXCR4 (Fig. 4B) cells with IL-8 (100 nM) also cross-desensitized RANTES (~55%) and SDF-1 (~40%)-induced GTPase activity in membranes (Fig. 4). Both RANTES and SDF-1 cross-inhibited IL-8-mediated GTPase activity by ~35 and ~45%, respectively. PMA (100 nM) heterologously desensitized the GTPase response to IL-8 (~35%), RANTES (~60%), and SDF-1 (~50%).

Cross-internalization of CCR5 and CXCR4—ACCR5 and ACXCR4 were treated with IL-8, RANTES, SDF-1, or PMA (100 nM), and receptor clearance from the cell surface was assessed by specific ligand binding. CXCR1 (Fig. 5, A and C), CCR5 (Fig. 5B), and CXCR4 (Fig. 5D) were homologously internalized by exposure of the cells to IL-8, RANTES, and SDF-1, respectively. IL-8 pretreatment cross-internalized CCR5 (Fig. 5B) but not CXCR4 (Fig. 5D). PMA pretreatment caused internalization of both CCR5 and CXCR4 (Fig. 5, B and D). RANTES, SDF-1, and PMA had no effect in CXCR1 internalization (Fig. 5, A and C).

Cross-phosphorylation of CCR5 and CXCR4—To assess the role of receptor phosphorylation in cross-internalization...
Cross-regulation of CCR5- and CXCR4-mediated HIV-1 Infection

Chemokines and chemokine receptors are redundant in that many chemokines activate more than one chemokine receptor, and many chemokine receptors are activated by multiple chemokines (8, 34). To date, the structural basis and biological significance of these redundancies remain unclear. Initial studies in our laboratory, however, provided evidence that chemokine receptors are capable of cross-regulating the functions of each other, thus limiting cellular responsiveness to chemo-

![Image](https://example.com/image.png)

**Table I**

Cross-desensitization among CCR5, CXCR4, and CXCR1 in transfected RBL cells

| Treatment | Peak Ca²⁺ mobilization | Percent desensitization |
|-----------|-------------------------|-------------------------|
| ACCR5-RBL |                         |                         |
| RANTES → RANTES | 622 ± 27 → 43 ± 7 | 93 |
| MIP-1β → MIP-1β | 571 ± 51 → 57 ± 10 | 94 |
| IL-8 → IL-8 | 593 ± 34 → 76 ± 13 | 87 |
| RANTES → IL-8 | 631 ± 43 → 301 ± 11 | 48 |
| IL-8 → RANTES | 576 ± 22 → 266 ± 17 | 58 |
| MIP-1β → IL-8 | 601 ± 14 → 348 ± 23 | 41 |
| IL-8 → MIP-1β | 589 ± 39 → 265 ± 19 | 56 |
| ACXCR4-RBL |                         |                         |
| SDF-1 → SDF-1 | 492 ± 18 → 44 ± 18 | 91 |
| IL-8 → IL-8 | 477 ± 45 → 69 ± 9 | 86 |
| SDF-1 → IL-8 | 455 ± 29 → 246 ± 20 | 52 |
| IL-8 → SDF-1 | 513 ± 22 → 297 ± 34 | 35 |

**Fig. 4.** Cross-desensitization of CCR5- and CXCR4-mediated GTPase activity. Double transfected RBL-2H3 cells expressing CXCR1 and either CCR5 (ACCR5) (A) or CXCR4 (ACXCR4) (B) were treated with IL-8 (100 nM), RANTES (100 nM), SDF-1 (100 nM), or PMA (100 nM) for 5 min. Membranes were prepared and assayed for agonist-stimulated GTP hydrolysis. The data are presented as percentage of control, which is the net maximal stimulation, obtained with untreated cells. Data shown are representative of one of three experiments performed in triplicate.

ACCR5 and ACXCR4 cells were labeled with ³²P and treated with IL-8 (100 nM), RANTES (100 nM), SDF-1 (100 nM), or PMA (100 nM). Cells were lysed, immunoprecipitated with the M2-FLAG (CCR5 and CXCR4)- or HA (CXCR1)-specific antibodies, and analyzed by SDS electrophoresis and autoradiography. The identities of the phosphorylated bands for the respective receptors (CXCR1, ~70 kDa; CCR5, ~40 kDa; and CXCR4, ~45 kDa) have been previously demonstrated (9, 10, 27). As shown in Fig. 6, CCR5 (A, lane 2) and CXCR4 (B, lane 2) were homologously phosphorylated by RANTES and SDF-1, respectively.

![Image](https://example.com/image.png)

**Fig. 5.** Cross-internalization of CCR5 and CXCR4. ACCR5 (A and B) and ACXCR4 (C and D) RBL cells (0.5 × 10⁶ cells/well) were treated with a 100 nM concentration of either IL-8, RANTES, SDF-1, or PMA at different times. Cells were then washed and assayed for ¹²⁵I-IL-8 (A and C), ¹²⁵I-RANTES (B), or ¹²⁵I-SDF-1 (D) binding. The values are presented as percentage of total, which is defined as the total amount of ¹²⁵I-ligand bound to control (untreated) cells. The experiment was repeated four times with similar results.

Role of Protein Kinase C in CCR5 Cross-internalization—Pretreatment of ACCR5 with the PKC inhibitor staurosporine (100 nM) partially inhibited RANTES-mediated CCR5 internalization (Fig. 7A) and phosphorylation (Fig. 7B). Cross-internalization and cross-phosphorylation by IL-8 as well as heterologous internalization and phosphorylation by PMA were totally inhibited by staurosporine (Fig. 7, A and B).

ΔCXCR1-mediated Cross-internalization and Cross-inhibition of CXCR4—The role of IL-8 in CXCR4 cross-internalization was further assessed by co-expressing a carboxyl terminus-deficient mutant of CXCR1, ΔCXCR1, along with CXCR4 (ΔCXCR4). The K₅₀ and Bₘ₅ₐₓ of ΔCXCR1 (2.1 ± 1.10 nm and 6898 ± 523 receptors/cell, respectively) were similar to those of CXCR1 (1.7 ± 0.33 nm and 7013 ± 311 receptors/cell, respectively). ΔCXCR1 mediated greater phosphoinositide hydrolysis (Fig. 8A), G-protein activation, secretion of β-hexosaminidase, and sustained Ca²⁺ response relative to CXCR1.² IL-8 pretreatment of ΔCXCR4 but not ΔCXCR1 cells resulted in cross-internalization of CXCR4 (Fig. 8B). In contrast to CXCR1, ΔCXCR1 activation also cross-inhibited CXCR4-mediated virus entry into MAGI cells (Fig. 8C).

**DISCUSSION**

Chemokines and chemokine receptors are redundant in that many chemokines activate more than one chemokine receptor, and many chemokine receptors are activated by multiple chemokines (8, 34). To date, the structural basis and biological significance of these redundancies remain unclear. Initial studies in our laboratory, however, provided evidence that chemokine receptors are capable of cross-regulating the functions of each other, thus limiting cellular responsiveness to chemo-
kines. The IL-8 receptor CXCR1 was shown to cross-desensitize responses to the CC receptor CCR1 at two levels: receptor/G-protein uncoupling via receptor cross-phosphorylation and inhibition of phospholipase C activity via phosphorylation of the enzyme, which diminishes its activation by G-protein (14, 35). The data herein describe another level of cross-regulation among chemokines that may have important consequences in their action: chemokine-mediated receptor cross-internalization. This contention is based on the following observations. First, IL-8 activation of CXCR1, but not CXCR2, cross-internalized

Fig. 6. Cross-phosphorylation of CCR5 and CXCR4. 32P-Labeled A) ACCR5 and B) ACXCR4 RBL cells were incubated for 5 min with or without stimulants as shown. Cells were then washed and assayed for 32P-RANTES binding as indicated in the legend of Fig. 5. The values are presented as percentage of total, which is defined as the total amount of 32P-RANTES bound to control (untreated) cells. The experiment was repeated twice with similar results.

Fig. 7. Effect of staurosporine on CCR5 cross-internalization and cross-phosphorylation. A) ACCR5 cells were incubated with and without staurosporine and treated with a 100 nM concentration of either IL-8, RANTES, or PMA for 60 min. Cells were then washed and assayed for 32P-RANTES binding as indicated in the legend of Fig. 5. The values are presented as percentage of total, which is defined as the total amount of 32P-RANTES bound to control (untreated) cells. The experiment was repeated twice with similar results. B) 32P-labeled ACCR5 cells were incubated with and without staurosporine for 5 min and then stimulated with a 100 nM concentration of either RANTES (lanes 3 and 4), IL-8 (lanes 5 and 6), or PMA (lanes 7 and 8). Cells were then washed and assayed for 32P-RANTES binding as indicated in the legend of Fig. 5. Two other experiments yielded similar results.

Fig. 8. A) for the generation of [3H]inositol phosphates, RBL cells were cultured overnight in the presence of [3H]inositol (1 Ci/ml) and stimulated with 100 nM IL-8 or SDF-1 for 10 min. Supernatant was used to determine the release of [3H]inositol phosphates. Data are represented as fold stimulation over basal. The experiment was repeated three times with similar results. B) ACXCR4 and ΔACXCR4 RBL cells were treated with a 100 nM concentration of IL-8 at different times and assayed for 32P-SDF-1 binding as described in the legend of Fig. 5. C) MAGI cells were transduced overnight with 10 ng of vesicular stomatitis virus glycoprotein-pseudotyped lentiviral vector encoding either CXCR1 or ΔCXCR1. Cells were treated with IL-8 (100 nM) and infected with X4-tropic virus, and luciferase activities in cell lysates were determined as described in the legend of Fig. 2.
CXC2 internalizes rapidly (90% after 2–5 min) and, as a consequence, does not mediate cross-regulatory signals (14, 29, 40). CXCR1, which is more resistant to internalization (50% after 20–40 min), mediated cross-phosphorylation and cross-desensitization of both CXC2 and CCR5 but cross-internalized and inhibited HIV-1 entry to CCR5 but not CXCR4 (Figs. 2, 4, and 5 and Table I) (14, 32, 33). ΔCXR1, however, which is far more resistant to internalization (10% after 60 min) and mediated greater cellular responses (i.e. phosphoinositide hydrolysis, Ca2+ mobilization), cross-internalized CXCR4 and inhibited T4-tropic virus entry (Fig. 8).2 This indicates a hierarchy in receptor-mediated cross-regulation that is directly correlated with the receptor resistance to desensitization, internalization, and, as a consequence, signaling time. Supporting that contention is that in monocytes isolated from mice deficient in β-arrestin-2 in which CXCR2 internalization is delayed (25% after 5 min) MGSA cross-desensitized Ca2+ mobilization to RANTES by ~50% relative to control or wild type mice (~90% after 5 min).3

CXCR1-mediated cross-desensitization and cross-internalization of CCR5 and CXCR4 as well as desensitization and internalization by PMA were inhibited by the PKC inhibitor staurosporine (Fig. 7 and data not shown). These results indicate that PKC may play a key regulatory role in the modulation of HIV-1 infection.

The resistance of CXCR4 to cross-internalization by CXCR1 may be explained in two ways. First, it could be that cross-regulation of CXCR4 is mediated via a PKC isoform different from that of CCR5, which requires greater second messenger production for its activation. Indeed ΔCXR1, which mediated greater phosphoinositide hydrolysis and Ca2+ mobilization, cross-internalized CXCR4. Second, previous studies in our laboratory have shown that PMA-induced CXCR4 internalization occurs via a mechanism distinct from receptor phosphorylation (9). It is likely that phosphorylation of an (other component(s) distal from the receptor/G-protein coupling is necessary for the PKC-mediated internalization and may require a higher level of second messenger production. Orsini et al. (11) have shown that a dileucine motif of the carboxyl terminus of the receptor that binds the adaptor protein-2 was necessary for the phosphorylation-independent internalization of the receptor.

In summary, these data demonstrate that the IL-8 chemokine can inhibit HIV-1 infection via CCR5 through activation of CXCR1 but not CXCR2. Inhibition of HIV-1 infection is not blocked by receptor desensitization alone but requires receptor internalization. CXCR4 is susceptible to CXCR1-mediated cross-desensitization but is resistant to cross-internalization. ΔCXR1 and PMA, which mediated greater cellular responses, cross-internalized and cross-inhibited CXCR4-mediated virus entry. This suggests that signaling through other chemokine receptors with stronger signal strengths may regulate the ability of CXCR4 as well as CCR5 to function as co-fusion proteins with CD4+. This observation presents new targets for therapeutic intervention against the infection and propagation of HIV-1.

An interesting finding in these studies is the importance of signal strength in cross-desensitization, cross-internalization, and inhibition of HIV-1 infectivity. Upon activation by IL-8, CXCR2 internalizes rapidly (90% after 2–5 min) and, as a consequence, does not mediate cross-regulatory signals as well as cross-desensitization of CXCR4, also inhibited HIV-1 infection through CXCR4. Activation of the formyl peptide receptor also cross-phosphorylated and cross-inhibited HIV-1 infection to CCR5 (16), but these chemoattractants are less commonly present at sites of inflammation than the chemokines. Chemokine production can be induced by many stimuli including cytokines, lipopolysaccharides, and viral products (39). Modulation of chemokine receptor internalization may therefore be a useful target for therapeutic intervention against HIV-1 infection.

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J. Biol. Chem. 2003, 278:15867-15873.
doi: 10.1074/jbc.M211745200 originally published online February 19, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M211745200

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