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The CXCR1 tail mediates β1 integrin-dependent cell migration via MAP kinase signaling

Ru Liu-Bryan [a], Salih Pay [a], Ingrid U. Schraufstatter [b], David M. Rose [a,*]

[a] Department of Medicine, Veterans Affairs Medical Center, University of California, San Diego, CA, USA
[b] Department of Vascular Biology, La Jolla Institute for Molecular Medicine, San Diego, CA, USA

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Abstract

In this study, we examined how IL-8 induces leukocyte migration on major β1 integrin ligands derived from the extracellular matrix protein fibronectin. We assessed individual contributions of signaling by IL-8 receptors by transfection of CXCR1 and CXCR2 into rat basophilic leukemia (RBL) cells and human monocytic THP-1 cells. CXCR1 expressing cells migrated on the fibronectin ligands for α4β1 and α5β1 integrins in response to IL-8, whereas CXCR2 expressing cells did not. RBL cells expressing the chimeric CXCR1 receptor containing the cytoplasmic tail of CXCR2 had greatly blunted migration, while cells expressing the CXCR2 chimera with the tail of CXCR1 had augmented migration. Last, inhibitors of p38 and JNK MAP kinases blocked IL-8-induced migration in CXCR1(+) cells. We conclude that IL-8 stimulated β1 integrin-mediated leukocyte migration on fibronectin through CXCR1 is dependent on the C-terminal cytoplasmic domain of CXCR1 and subsequent p38 and JNK MAPK signaling.

Keywords: Chemokine receptors; Cell migration; Interleukin-8; Leukocytes

The migration of leukocytes from the blood into peripheral tissues is central to both the initiation and subsequent organization of inflammation [1,2]. Leukocyte entry into tissues requires migration across the endothelial monolayer and basement membrane, processes stimulated by a variety of chemotaxins, including chemokines, and modulated by numerous adhesion molecules such as selectins and integrins [3,4]. The importance of chemokines in directing β2 integrin-dependent leukocyte arrest and diapedesis across the endothelium is well established [5,6]. Though previous studies with neutrophils have suggested that β1 integrins are more involved than β2 integrins in the migration of leukocytes through the extracellular matrix (ECM) [7], chemokine-regulated movement of leukocytes through the ECM is less well defined.

IL-8 and related members within a subgroup of the CXC chemokine family have been implicated in the pathogenesis of diverse acute and chronic inflammatory diseases [8,9]. In humans, IL-8, GRO chemokines, ENA-78, and several other closely related CXC chemokines bind to and signal through the promiscuous G protein-linked 7-membrane spanning receptor CXCR2 [10,11]. A highly homologous but functionally distinct receptor with greater specificity, CXCR1, binds IL-8 with high affinity, but does not appear to bind most other CXCR2 ligands [12,13]. The structural differences between CXCR1 and CXCR2 responsible for the differential functions of the two receptors are not known. The two receptors are highly homologous at the amino acid level except at the N-termini, fourth transmembrane domains, and C-termini [10,11]. The N-terminus is critical for ligand binding, and differences in this region are implicated in ligand specificity and affinity modulation [14]. The functional importance of heterogeneity at the

* Corresponding author. Fax: +1 858 552 7425.
E-mail address: drose@vapop.ucsd.edu (D.M. Rose).

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C-terminus has not been fully defined. This region is not thought to be critical in ligand binding specificity or receptor affinity [15,16].

Since CXCR1 and CXCR2 are often co-expressed on cells, it has been difficult to determine if the receptor subtypes have distinct signal and functional properties. Antibody inhibition studies have demonstrated that CXCR1 and CXCR2 signaling can initiate similar increases in intracellular calcium ions and elastase release in neutrophils [17,18]. However, IL-8-induced cell migration, phospholipase D activation, and superoxide anion generation are predominantly mediated by CXCR1 [19–21]. It is not clear whether these differential responses are due to distinct primary signal transduction events for CXCR1 and CXCR2 or whether secondary events and/or cross talk between the two receptors are responsible for the CXCR1-specific migratory behavior. Secondary events might include priming of CXCR1 by CXCR2 activation [22], faster phosphorylation and down-regulation of the CXCR2 [23,24], and increased affinity of N-terminally truncated IL-8 for the CXCR1 following cleavage by neutrophil-derived gelatinase B [25]. Furthermore, it is uncertain whether preferential chemotaxis mediated by CXCR1 applies to leukocytes other than neutrophils and whether integrin-dependent migration through ECM is also preferentially mediated by CXCR1.

In the present study, we examined the differential role of CXCR1 and CXCR2 in mediating IL-8-induced leukocyte migration on two peptide domains of the major ECM constituent fibronectin (CS-1, a ligand of integrin $\alpha4\beta1$; the fibronectin cell attachment domain, a ligand of the integrin $\alpha5\beta1$). We report that IL-8-induced $\beta1$ integrin-dependent leukocyte migration on fibronectin is mediated through CXCR1 and not CXCR2, and that the C-terminal cytoplasmic domain of CXCR1, and p38 and JNK mitogen-activated protein kinase (MAPK) signaling mediate this preferential migration via CXCR1.

Methods

Reagents. Monoclonal anti-human CXCR1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and a rabbit polyclonal anti-human CXCR2 was obtained as previously described [24]. The MEK inhibitor PD98059, p38 inhibitor SB203580, and JNK inhibitor SP600125 were purchased from Calbiochem–Novabiochem (San Diego, CA). The cDNA encoding the CS-1 region of fibronectin fused to GST was a gift of Dr. Jeffrey W. Smith (Burnham Institute, La Jolla, CA). The expression and purification of these fusion proteins has been previously described [26]. The fragment of fibronectin containing the type III repeats 9–11 fused to GST was expressed and purified as described [27]. Neutralizing antibodies to CXCR1 and CXCR2 were generously provided by Dr. K.J. Kim (Genetech, San Francisco, CA). Anti-$\alpha4$ integrin antibody (HP2/1) and anti-$\alpha5$ integrin antibody (SAM1) were purchased from Immunotech (Westbrook, ME)

Construction of chimeric CXCR1 and CXCR2. The cDNAs for human CXCR1 and CXCR2 in the plasmid pSFFV.NEO [24] were subcloned into the *Eco*RI site of pcDNA3.1 (−) (Invitrogen, Carlsbad, CA) containing a previously deleted *Xba*I site. A silent *Xba*I site was introduced by QuickChange site-directed mutagenesis (Stratagene, La Jolla, CA) in the vicinity of the coding region for alanine286–leucine287 of CXCR1 and the conserved region of CXCR2. Primers to introduce such mutations were as follows: CXCR1 sense 5′-CATC GGGCGGCTCTAGATGCCACTGAGATTCTG-3′; CXCR1 antisense 5′-CAGAATCTCAGTGGCATCAGAGCCCGGCCGATG-3′; CXCR2 sense 5′-CACATCGACCGGGCTCTAGATGCCACCGAG ATTCTG-3′; CXCR2 antisense 5′-CAGAATCTCGGTGGCATCTA GAGCCCGGTCGATTGT-3′. Each construct was cut with *Nhe*I and *Xba*I. The resulting fragment encoding the N-terminal 285 animo acids of CXCR1 was ligated to the plasmid encoding the C-terminal 66 amino acids of CXCR2 (designated CXCR1T2), and the fragment encoding the N-terminal 294 amino acids of CXCR2 was ligated into the plasmid encoding the C-terminal 65 amino acids of CXCR1 (designated CXCR2T1). All constructs were sequenced to verify that no extraneous mutations were introduced during mutagenesis.

Cells. RBL2H3 and THP-1 cells were obtained from ATCC (Rockville, MD) and grown in RPMI-1640 supplemented with 10% FCS, 1% glutamine, 50 U/ml penicillin, and 50 mg/ml streptomycin. The generation of RBL cells expressing CXCR1 and CXCR2 has been previously described [24]. THP-1 cells expressing CXCR1 and overexpressing CXCR2, and RBL cells expressing chimeric receptors CXCR1T2 and CXCR2T1 were generated by similar means. Briefly, cells (1×10^7) were transfected with cDNAs (60 μg) by electroporation using an ECM630 electroporator (BTX, San Diego, CA). Cells were grown in the presence in G418 1 mg/ml for 3 weeks. Afterward, cells were stained for CXCR1 or CXCR2 and sorted using a FACStarplus flow cytometer (BD Biosciences, Mountain View, CA) into 96-well tissue culture-treated plates (Corning, Corning, NY). Isolated clonal lines were subsequently reassayed for CXCR1 and CXCR2 expression, and used for analysis.

Calcium mobilization. To detect changes in intracellular calcium, RBL2H3 cells were labeled for 30 min with 5μM indo-1-AM (Molecular Probes). Fluorescent labeled cells (2×10^5) were warmed to 37 °C for 2 min in a stirred cuvette containing 200 μl of modified Gey's buffer (140 mM NaCl, 5 mM KCI, 1.9 mM KH_2PO_4, 0.5 mM $MgCl_2$, 1.1 mM Na_2HPO_4, 1.5 mM Ca_2Cl, 10 mM Hepes, and 5.5 mM glucose, pH.7.4), the stimulus was added, and the emission ratio at 400/490 nm was followed kinetically on an SLM 8000 fluorometer as described previously [28]. Calcium concentrations were calculated as described in [20].

Actin polymerization. For the determination of F-actin, RBL2H3 cells (10^7 cells/ml) were stimulated with 100 ng/ml IL-8 for the indicated times at 37 °C. Cell aliquots were pipetted into a mixture of formaldehyde, lysophosphatidic acid, and FITC-phalloidin (Sigma, St. Louis, MO) as previously described [22], and the mean fluorescence of the cell population was detected by flow cytometric analysis on a FACScan instrument (BD Biosciences, Mountain View, CA).

Receptor surface expression. The flow cytometric analysis of receptor expression with specific anti-receptor antibodies was performed as previously described [23] using a monoclonal anti-CXCR1 antibody and a polyclonal anti-CXCR2 antibody followed by staining with FITC-conjugated secondary antibody (Biosource International, Camarillo, CA). Bound antibody was detected using a FACS Caliber flow cytometer (BD Biosciences, Mountain View, CA) and analyzed using CellQuest software.

Cell migration assay. Cell migration was assayed in a modified Boyden chamber assay system. Transwells (Costar, Corning), polycarbonate membranes containing 5.0 μm pores, were incubated with GST-CS-1 or GST-9,11 fibronectin fusion proteins (5 μg/ml) in 0.1 M $NaHCO_3$, pH 8.0, overnight at 4 °C. Membranes were blocked with 2% BSA in PBS for 30 min at room temperature. We added 2.0×10^5 cells in RPMI-1640 with 10% FCS to the top chamber. Chemokines at the indicated concentrations were added to the bottom chamber. Cells were allowed to migrate for 4–8 h at 37 °C. Cells in the bottom chamber were enumerated with a hemocytometer.

Results

Generation and characterization of leukocyte cell lines expressing CXCR1 and CXCR2

To study the functional and signaling differences between CXCR1 and CXCR2, we developed stable cell lines expressing one or both of these receptors. Rat basophilic leukemia (RBL) cells express neither CXCR1 nor CXCR2. Subsequently, RBL cells were transfected with cDNAs encoding human CXCR1 or CXCR2 and stable lines isolated by FACS (Fig. 1A). The human monocytic cell line THP-1 expresses endogenous CXCR2 but not CXCR1 (Fig. 1B). These cells were transfected with either control DNA or human CXCR1 or CXCR2. Cells were sorted by FACS to isolate high receptor expressing clones.

To determine if the CXCR1 and CXCR2 were functional in these cells, we first questioned if chemokines would stimulate increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$). In RBL-CXCR1 cells, IL-8 stimulated a transient raise in $[Ca^{2+}]_i$ (Fig. 1C), while GRO-α, a CXCR2-selective agonist, failed to stimulate Ca^{2+} fluxes in these cells. In RBL-CXCR2 cells, transient increases in $[Ca^{2+}]_i$ were stimulated by both IL-8 and GRO-α (Fig. 1C). In THP-1-Neo cells, expressing endogenous CXCR2, IL-8 also stimulated an increase in $[Ca^{2+}]_i$ (data not shown). Increases in $[Ca^{2+}]_i$ stimulated by IL-8 were augmented in THP-1-CXCR1 and THP-1-CXCR2 cells. In addition, we questioned if IL-8 could stimulate F-actin polymerization in cells expressing CXCR1 or CXCR2. In both RBL-CXCR1 and RBL-CXCR2 cell lines, IL-8 stimulated a rapid increase in actin polymerization (Fig. 1D), albeit to a lesser degree in cells expressing CXCR2. Thus, based on changes in intracellular Ca^{2+} levels and F-actin polymerization, the transfected chemokine receptors were functional in these cells.

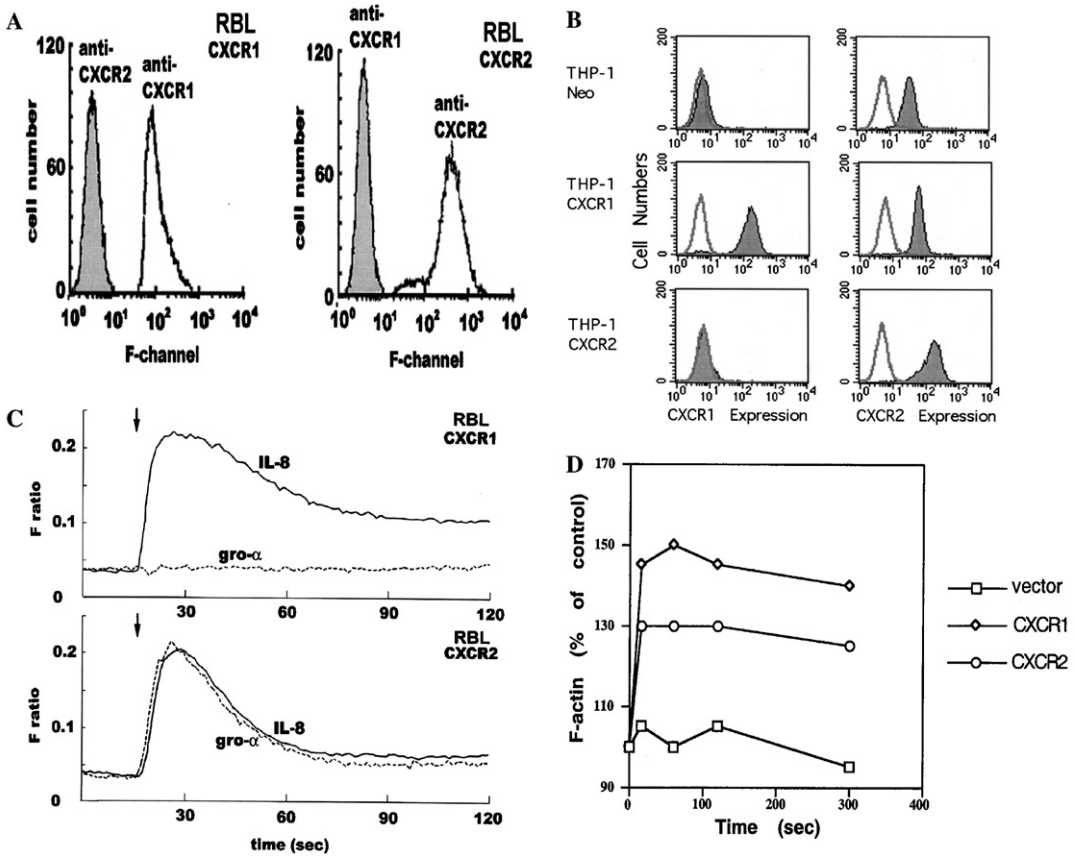

Fig. 1. Surface expression and function of CXCR1 and CXCR2 on transfected RBL and THP-1 cells. (A) RBL cells stably transfected with CXCR1 (left panel) or CXCR2 (right panel) were stained with monoclonal anti-CXCR1 or polyclonal anti-CXCR2. Bound antibody was detected with FITC-conjugated secondary antibodies by flow cytometry. (B) THP-1 cells stably transfected with vector control DNA (THP-1-neo), CXCR1 (THP-1-CXCR1), or CXCR2 (THP-1-CXCR2) were stained with isotype control antibody (open histogram) or anti-CXCR1 or anti-CXCR2 (closed histograms). Bound antibody was detected with FITC-conjugated secondary antibody. (C) RBL cells expressing CXCR1 (top panel) or CXCR2 (lower panel) were loaded with indo-1-AM. Cells were treated with IL-8 (solid line) or GRO-α (broken line) at times indicated by the arrows. Changes in free calcium are expressed as fluorescence units. (D) RBL cells expressing CXCR1 or CXCR2 were treated with IL-8 and F-actin polymerization detected by FITC-phalloidin staining as measured by flow cytometry. Results are representative of three separate experiments.

IL-8 preferentially stimulated β1 integrin-dependent migration on fibronectin through its interaction with CXCR1

We next examined the migratory behavior of cells expressing CXCR1 or CXCR2. Using transwells coated with the CS-1 fragment of fibronectin as the ligand for the integrin α4β1, parental RBL and RBL-CXCR2 cell lines did not migrate in response to IL-8 even at concentrations as high as 500 ng/ml (Fig. 2). In contrast, RBL cells expressing CXCR1 migrated in response to IL-8. The migration on fibronectin peptides was dose-dependent and maximal at an IL-8 concentration of 100 ng/ml. Similar results were obtained with THP-1 cells. THP-1-neo cells, expressing endogenous CXCR2, as well as cells overexpressing CXCR2 did not migrate in response to IL-8, whereas cells transfected with CXCR1 did (Fig. 2). We also tested two other chemokines, GRO-α and ENA-78, which are selectively ligands for CXCR2. As shown in Fig. 3A, neither GRO-α nor ENA-78 at 10 ng/ml stimulated cell migration of RBL or THP-1 cells expressing CXCR2. Furthermore, GRO-α and ENA-78 at concentrations as high as 500 ng/ml also failed to stimulate migration of cells expressing CXCR2 (data not shown). In addition, the lack of migration of CXCR2 expressing cells was not a clonal artifact as multiple clones of RBL and THP-1 cells expressing CXCR2 failed to migrate in response to IL-8. In addition, migration of RBL-CXCR1 and THP-1-CXCR1 cells stimulated by IL-8 was blocked by antibodies to CXCR1 but not CXCR2 (data not shown). Thus, in these cells, CXCR1 was the receptor subtype responsible for IL-8-induced migration on CS-1.

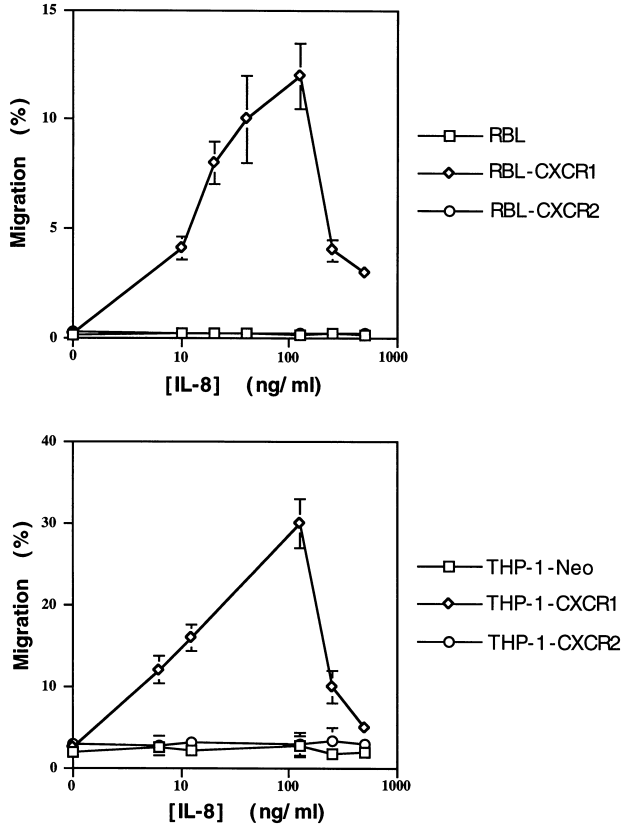

Fig. 2. Migration of RBL and THP-1 cells expressing CXCR1 and/or CXCR2 in response to IL-8. Cell migration was measured in a transwell system as described in the methods section. Cells (RBL top panel; THP-1 bottom panel) were added to the top chamber of the transwells coated with the CS-1 fragment of fibronectin, and the indicated concentration of IL-8 added to the bottom chamber. After 8 h (RBL cells) or 4 h (THP-1 cells), the cells in the bottom chamber were collected and enumerated with a hemocytometer. Migration is expressed as a percent of input cells. Results are means ± SEM of three separate experiments.

We next tested whether CXCR1 would mediate migration on more than one substrate for β1 integrin. Transwells were coated with a fragment of fibronectin containing the ninth through eleventh type III repeats (cell attachment domain, or 9,11 fragment) as the ligand for α5β1. Cells expressing CXCR1 migrated on the 9,11 fragment in response to IL-8, but not GRO-α or ENA-78 (Fig. 3A). In contrast, cells expressing CXCR2 did not migrate in response to either of these chemokines (Fig. 3A) even at concentrations as high as 500 ng/ml (data not shown). For both RBL-CXCR1 and THP-1-CXCR1 cells, the migration on the CS-1 fragment of FN was blocked with an anti-α4 antibody and that on the 9,11 fragment of FN by an anti-α5 antibody (Fig. 3B). Thus, in this system, IL-8 stimulated migration was β1-integrin dependent, and the lack of chemokine-induced migration mediated by CXCR2 was not limited to a single combination of β1 integrin and ligand usage.

The CXCR1 cytoplasmic tail is critical for mediating IL-8-directed β1 integrin-dependent cell migration

CXCR1 and CXCR2 show the most sequence diversity at their N- and C-termini, and fourth transmembrane domains. Since the divergence in the N-termini has been linked to differences in ligand specificity, we questioned whether divergence at the C-termini might be important in differential migratory responses mediated by the two receptors. To test this, we made chimeric receptors by swapping the C-terminal domains and established stable RBL cell lines expressing these chimeras (Fig. 4A). The substitution of the cytoplasmic domain of CXCR1 with that of CXCR2 greatly attenuated IL-8-induced cell migration (Fig. 4B). Conversely, the replacement of the C-terminus of CXCR2 with that of CXCR1 transformed the CXCR2 into a receptor capable of mediating chemotaxis induced by IL-8 (Fig. 4B). These results implicate the C-terminus cytoplasmic domain of CXCR1 as critical for mediating IL-8-induced migration through CXCR1.

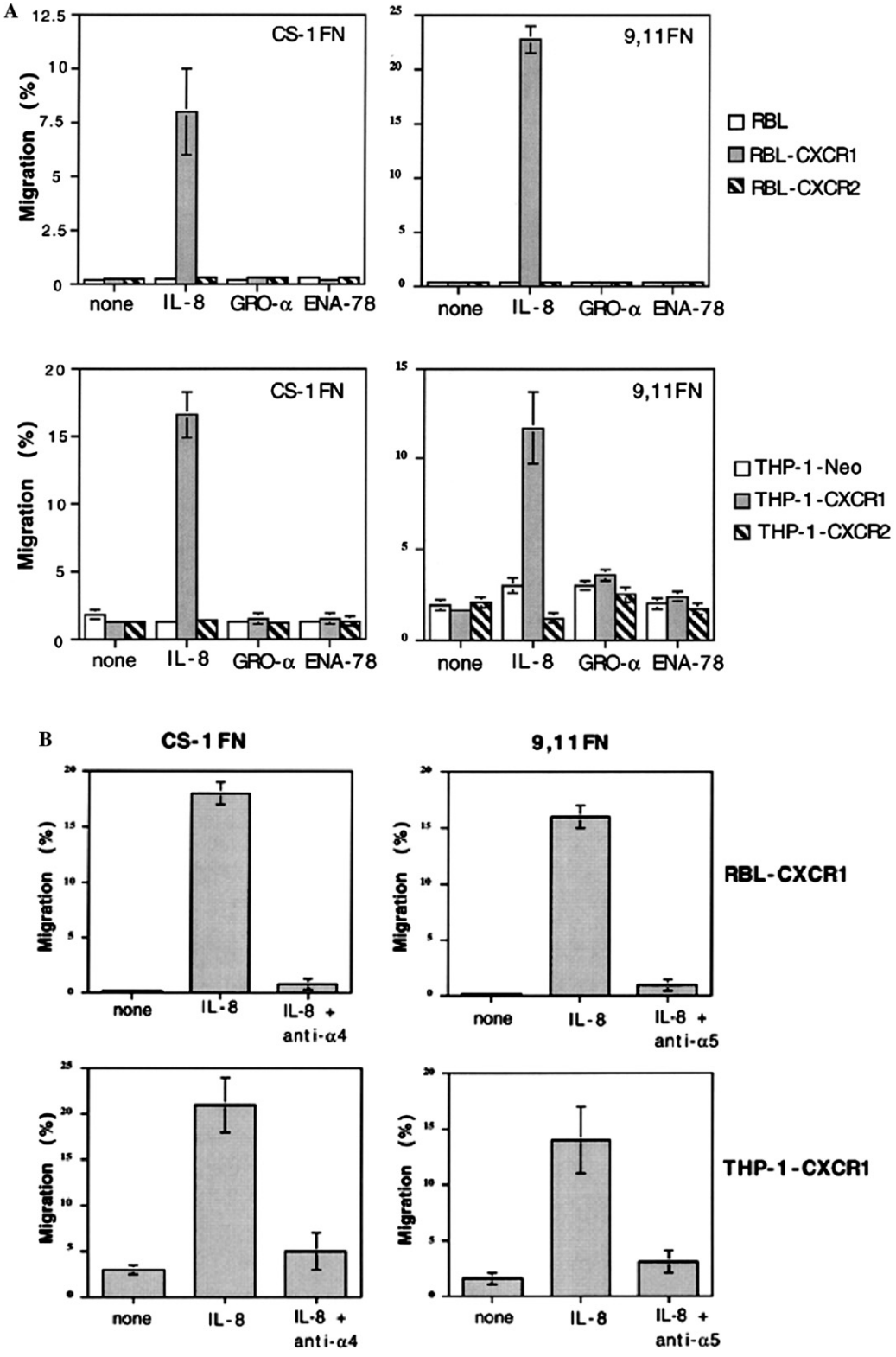

Fig. 3. Migration of RBL and THP-1 cells expressing CXCR1 and/or CXCR2 on fibronectin ligands for α4β1 and α5β1 integrins. Migration assays were performed as described above. (A) RBL (top panel) and THP-1 (bottom panel) cells expressing CXCR1 and/or CXCR2 were added to the top chamber of the transwells coated with either CS-1 fragment (ligand for α4β1 integrin) (left panels) or the 9,11 type III repeat (ligand for α5β1 integrin) (right panels) fragment of fibronectin (FN) and chemokines (10 ng/ml) added to the bottom chamber. Cells migrating to the bottom chamber were enumerated with a hemocytometer and expressed as a percent of input cells. (B) Cells were treated with 10 μg/ml of function blocking anti-α4 antibody (HP2/1)(left panels) or anti-α5 antibody (SAM1) (right panels) for 30 min prior to performance of migration assays. Results are means ± SEM of three separate experiments.

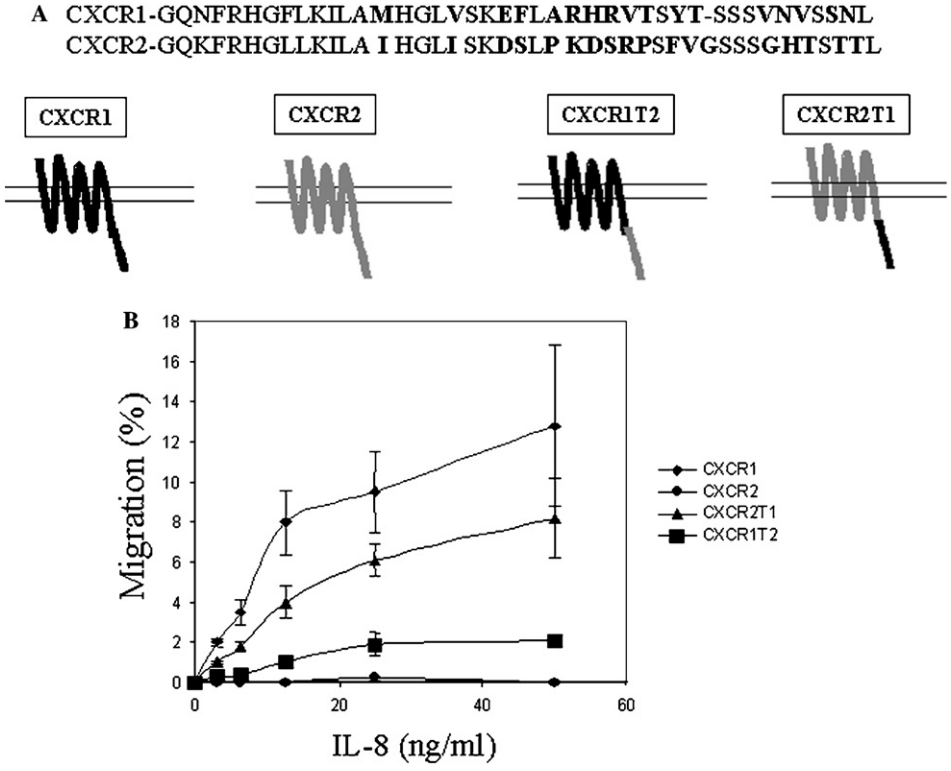

Fig. 4. The CXCR1 cytoplasmic tail is critical for mediating IL-8-induced β1 integrin-dependent cell migration. (A) Amino acid sequence alignment of the last 42 amino acids of the C-terminal domains of CXCR1 and CXCR2. Divergence in amino acid sequence is bolded. Schematic representations of chimeric receptors generated by swapping the C-terminal domains between CXCR1 and CXCR2 are shown (see Methods for details on chimeric construction). (B) RBL cells stably expressing CXCR1, CXCR2 or the chimeric receptors CXCR1T2 or CXCR2T1 were assessed in migration assays as performed above. Cells were added to the top chamber of transwells coated with the CS-1 fragment of fibronectin, and IL-8 added to the bottom chamber. Cells that migrated to the bottom chamber were enumerated with a hemocytometer and expressed as a percent of input cells. Results are means ± SEM of three separate experiments.

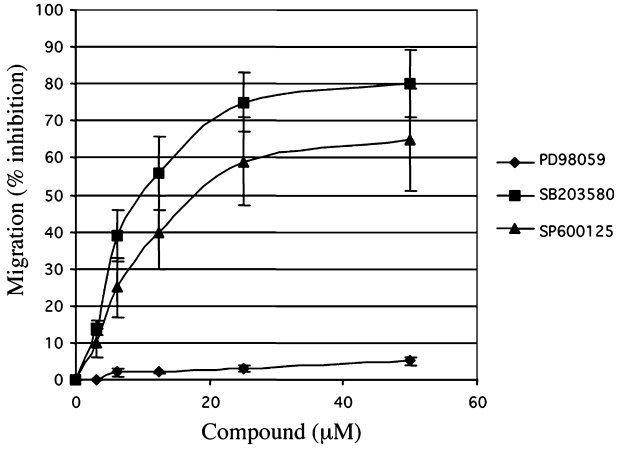

Fig. 5. IL-8 acting through CXCR1 preferentially stimulates cell migration via p38 and JNK MAP kinase-dependent signaling. RBL cells expressing CXCR1 were treated with the indicated concentrations of the p38 inhibitor (SB203580), JNK inhibitor (SP600125), or the MEK inhibitor (PD98059) and allowed to migrate across transwells coated with the CS-1 fragment of fibronectin. In all cases, IL-8 was used at a concentration of 10 ng/ml. Migration is expressed as percent inhibition relative to no inhibitor treatment. Results are means ± SEM of three separate experiments.

IL-8 stimulated cell migration preferentially through CXCR1 via p38 and JNK MAPK-dependent signaling

Results from the migration studies suggested that one or more signaling events needed for effective migration stimulated by IL-8 were not initiated by CXCR2 signaling. To determine if ERK, p38, or JNK MAPK signaling was involved in IL-8-induced chemotaxis, we treated cells with the MEK inhibitor PD98059, p38 inhibitor SB203580, and JNK inhibitor SP600125. The p38 and JNK inhibitors markedly blocked IL-8-induced migration of RBL-CXCR1 cells with an $IC_{50} \sim 15\ \mu M$ (Fig. 5). Similarly, the p38 inhibitor blocked IL-8-induced migration of THP-1-CXCR1 cells (data not shown). In contrast, the MEK inhibitor had no effect on IL-8 stimulated cell migration (Fig. 5). Collectively, IL-8 stimulated cell migration mediated by CXCR1 was dependent on p38 and JNK MAPK signaling.

Discussion

Subsequent to crossing the endothelial monolayer, the movement of leukocytes through the ECM is critical

to the organization of the inflammatory response. Herein, we report that IL-8-induced β1 integrin-dependent leukocyte migration on two specific peptide domains of the ECM molecule fibronectin was mediated predominantly via CXCR1 but not CXCR2 signaling. This differential migratory response was largely mediated by differences in the C-terminal cytoplasmic domains of these two receptors. In addition, IL-8-induced migration was dependent on p38 and JNK MAPK signaling. The findings in this study took particular advantage of parental CXCR1(−), CXCR2(−) RBL cells, in which heterologous expression of CXCR1, but not CXCR2, resulted in the ability of IL-8 to stimulate cell migration. Similarly, in THP-1 cells, endogenous CXCR2 expression was insufficient to transduce IL-8-induced migration, whereas CXCR1 transfection rendered these cells capable of IL-8-induced migration. Taken together, our findings indicated that IL-8 preferentially induces β1 integrin-dependent cell migration through CXCR1 via p38 and JNK MAPK-dependent signaling pathway.

Leukocyte migration via β1 integrins driven by IL-8 is mediated preferentially by CXCR1 rather than CXCR2. Previous work using primary neutrophils, but limited technically by the use of IL-8 receptor-selective neutralizing antibodies, has also suggested that IL-8 preferentially stimulates chemotaxis through CXCR1 [20]. But the molecular approach used in this study to ascertain that IL-8 directs β1 integrin-dependent migration on fibronectin peptides through CXCR1 was unique, and reproducible in two different cell systems from distinct species (RBL and THP-1). The CXCR2 receptors were demonstrated to be functional in both these cell types. Thus, the lack of CXCR2 mediated migration in RBL cells was not due to species incompatibility with the human CXCR2. Our results differ from those reported by Richardson et al. [16], who found that CXCR1 and CXCR2 expressed in RBL cells drive equivalent IL-8-induced migration. The reason for this difference may stem from the form of migration studied. We specifically examined β1 integrin-dependent migration using defined ligands for α4β1 and α5β1 integrins. It is conceivable that migration mediated by other adhesion molecules may be driven by CXCR2 signaling. This may have biological importance as migration in subendothelial tissue which is rich in extracellular matrix proteins, ligands for β1 integrin, may be driven by CXCR1 signaling, while migration in other location may be mediated by CXCR2.

Signaling and functional differences mediated by CXCR1 and CXCR2 may also be influenced by the cellular context in which the receptors are expressed. Though IL-8 also stimulates migration of neutrophils predominant via its interaction with CXCR1, in Jurkat T leukemic cells, CXCR1 and CXCR2 both can independently initiate cell migration [29]. Thus, CXCR2 is not likely to be intrinsically incapable of signaling cell migration; instead, the receptor almost certainly requires a cellular environment with the necessary signaling machinery needed to support cell migration. Indeed, in lung microvascular endothelial cells, IL-8 stimulated migration preferentially through CXCR2 rather than CXCR1 [30]. Essentially, the cellular context in which the two IL-8 receptors are expressed appears to markedly influence functions mediated by IL-8 and its family members. Many cell types including neutrophils express both CXCR1 and CXCR2 abundantly [10–13], suggesting the possibility of cooperativity between CXCR1 and CXCR2 in IL-8-induced cell migration. But this hypothetical mechanism for migration on fibronectin peptides was excluded from this study. First, transfection of CXCR1 but not CXCR2 into parental CXCR1 negative and CXCR2 negative RBL cells allowed for cell migration. Second, anti-CXCR1 antibodies, but not CXCR2 antibodies, blocked migration in both RBL and THP-1 cells. Third, the lack of CXCR2-directed cell migration was not limited to the ligand IL-8 as two other selective CXCR2 ligands, GRO-α and ENA-78, also failed to stimulate migration on fibronectin ligands in both RBL and THP-1 cells. Collectively, these results indicated that IL-8 signaling through CXCR1 preferentially triggered leukocyte migration on fibronectin peptides.

The C-terminal cytoplasmic domains of CXCR1 and CXCR2 are critical in determining the differential ability of the receptors to signal IL-8-induced cell migration on fibronectin. Exchanging the C terminals of CXCR1 and CXCR2 largely recapitulated the migratory propensities of the receptors; namely, replacing the C-terminal domain of CXCR1 with CXCR2 inhibited migration, while the converse promoted IL-8-induced cell migration. However, the reversal of phenotype by domain swap was not complete, suggesting that other regions of the receptors are also involved. The fourth transmembrane domain is a logical candidate as this region also shows a high degree of sequence divergence between CXCR1 and CXCR2. Cell migration studies using C-terminal truncations of CXCR1 also lend credence to the importance of this domain in regulating IL-8-induced cell migration [16]. The biochemical mechanism mediating the differential signaling between the cytoplasmic domains of CXCR1 and CXCR2 is not known. Certainly, receptor phosphorylation of CXCR1 and CXCR2 has been shown to modulate receptor desensitization and internalization [31,23,16]. Many of the serine residues in the C-terminal tail of the two receptors are conserved. This makes differential serine phosphorylation of an unlikely mechanism to explain the differential migratory properties. However, CXCR1 does have two unique threonine residues in the C-terminal tail at positions 346 and 349, which are not found in CXCR2. It is unclear whether these residues are phosphorylated, but we recently have found if these threonine residues are

mutated it reduced IL-8-induced inorganic phosphate uptake in chondrocytes [32]. Whether these sites also play an important role in cell migration is currently under investigation.

IL-8-induced cell migration mediated by CXCR1 requires p38 and JNK MAPK signaling, but not ERK. This conclusion is based on the inhibition of IL-8-induced cell migration in RBL-CXCR1 cells with selective p38 and JNK MAPK inhibitors, but not a MEK inhibitor, the immediate upstream kinase responsible for ERK activation. This is in agreement with recent studies done in neutrophils in which a p38, but not a MEK, inhibitor blocked IL-8-induced chemotaxis [33]. Our current work expands upon these findings to demonstrate the dual necessity of both p38 and JNK MAPK in IL-8-induced migration. In a number of cell systems, the activation of one or more MAPK family members has been implicated in regulating cell migration [34]. The lack of involvement of ERK activation in IL-8-induced migration was not a matter of failure of IL-8 to trigger ERK activation as we observed activation of all three MAPK by IL-8 in these cells.

IL-8, CXCR2-binding chemokines related to IL-8, and CXCR2 signaling mediate not only acute inflammatory responses but also chronic organized inflammation [35,36]. However, IL-8 and CXCR2-mediated responses also are major mediators of host defense [37]. In this context, the differential nature of CXCR1 and CXCR2 functional responses reinforced by this study has potentially significant implications for therapies to control IL-8-mediated inflammation. Specifically, the results of this study point to the potential means for suppressing the organization of chronic inflammatory responses through CXCR1 inhibition without impeding constitutive host defense functions by interfering with activation of CXCR2.

Acknowledgments

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