Interleukin-8: novel roles in human airway smooth muscle cell contraction and migration

Vasanthi Govindaraju,1 Marie-Claire Michoud,1 Mustafa Al-Chalabi,1 Pasquale Ferraro,2 William S. Powell,1 and James G. Martin1

1Seymour Heisler Laboratory of the Montreal Chest Institute Research Center and Meakins-Christie Laboratories, McGill University, Montreal; and 2University of Montreal Hospital Center, Montreal, Quebec, Canada

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Interleukin-8: novel roles in human airway smooth muscle cell contraction and migration. Am J Physiol Cell Physiol 291: C957–C965, 2006.  First published July 5, 2006; doi:10.1152/ajpcell.00451.2005.—In patients with cystic fibrosis (CF) and asthma, elevated levels of interleukin-8 (IL-8) are found in the airways. IL-8 is a CXC chemokine that is a chemoattractant for neutrophils through CXCR1 and CXCR2 G protein-coupled receptors. We hypothesized that IL-8 acts directly on airway smooth muscle cells (ASM) in a way that may contribute to the enhanced airway responsiveness and airway remodeling observed in CF and asthma. The aim of this study was to determine whether human ASM (HASMC) express functional IL-8 receptors (CXCR1 and CXCR2) linked to cell contraction and migration. Experiments were conducted on cells harvested from human lung specimens. Real-time PCR and fluorescence-activated cell sorting analysis showed that HASMC expressed mRNA and protein for both CXCR1 and CXCR2. Intracellular Ca2+ concentration ([Ca2+]i) increased from 115 to 170 nM in response to IL-8 (100 nM) and decreased after inhibition of phospholipase C (PLC) with U-73122. On blocking the receptors with specific neutralizing antibodies, changes in [Ca2+]i were abrogated. IL-8 also contracted the HASMC, decreasing the length of cells by 15%, and induced a 2.5-fold increase in migration. These results indicate that HASMC constitutively express functional CXCR1 and CXCR2 that mediate IL-8-triggered Ca2+ release, contraction, and migration. These data suggest a potential role for IL-8 in causing abnormal airway structure and function in asthma and CF.

INTERLEUKIN-8 (IL-8) is a member of the CXC chemokine subfamily of cytokines and may evoke the migration of neutrophils (26), monocytes (40), and eosinophils (41) to the sites of inflammation, injury, or infection. In neutrophils, IL-8 induces shape change, exocytosis of stored proteins, and the respiratory burst, resulting in the release of superoxide anion and hydrogen peroxide (5). IL-8 is also involved in a wide variety of physiological and pathological processes, including host defense against bacterial infection, angiogenesis, arteriosclerosis, and autoimmune disorders of skin, bones, and joints (20). In addition, elevated concentrations of IL-8 are found in sputum, bronchoalveolar lavage fluid, and bronchial tissues of subjects with pulmonary diseases such as severe asthma, occupational asthma (18), cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) (50), bronchitis, acute respiratory distress syndrome, and idiopathic pulmonary fibrosis. The potential sources of IL-8 in the airways include neutrophils, airway epithelial cells, fibroblasts, mast cells, and macrophages (8, 31).

IL-8 mediates its effects through the activation of specific G protein-coupled receptors (27). Two subtypes of receptors have been described, CXCR1 (22) and CXCR2 (33). CXCR1 principally binds IL-8 with high affinity, whereas CXCR2 binds to IL-8 and other CXC chemokines, such as GRO-α, GRO-β, GRO-γ, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 (1). Both CXCR1 and CXCR2 undergo homologous and heterologous desensitization (2). The internalization mechanisms are different for CXCR1 and CXCR2 (6). Because desensitization and internalization of CXCR2 occur faster than desensitization of CXCR1 at a lower concentration of CXCL8, CXCR1 may play a important role in settings such as acute inflammation, where higher levels of IL-8 may be present (9).

In neutrophils, IL-8 activates phospholipase C (PLC) (49) and mobilizes intracellular Ca2+. Similar actions in human airway smooth muscle cells (HASMC) would be expected to result in contraction. CF and chronic asthmatic patients have reversible narrowing of the airways in response to nonspecific irritants and inflammatory mediators (4, 25). The cause of the airway hyperresponsiveness in these conditions is still a matter for debate. The intrinsic responsiveness of airway smooth muscle (ASM) to contractile agonists is enhanced by cytokines such as IL-1β, IL-13, IL-5, IL-10, TNF-α, and granulocyte-macrophage colony-stimulating factor (3). Morphological studies of the airways show chronic infection and inflammation, damage to the airway epithelium, and increased thickness of the ASM layer (14, 15, 43). An increase in ASM mass could contribute to airway hyperresponsiveness by providing an increase in force required to overcome the intrinsic impedances that normally limit airway narrowing. Recently, cellular migration of myofibroblasts from subepithelial sites of proliferation in response to agonists has been proposed as one of the mechanisms for the increase in ASM mass observed in asthma and other diseases associated with ASM hyperplasia (24, 32). Given the signal transduction pathways activated by IL-8, an excess of IL-8 in the airways might be expected to stimulate ASM functions, including contraction and migration. We hypothesized that IL-8 acts through specific CXC receptors to cause the activation of HASMC with subsequent contraction and migration. These effects on smooth muscle may promote excessive airway narrowing and remodeling, pathophysiological processes associated with airway diseases in which IL-8 is frequently present in increased amounts.

Address for reprint requests and other correspondence: J. G. Martin, Meakins-Christie Laboratories, Dept. of Medicine, McGill Univ., 3626 St Urbain, Montreal, Quebec, Canada H2X 2P2 (email: james.martin@mcgill.ca).

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Primary cultures of HASMC were prepared from surgical specimens as previously described (19, 34). Segments of lobar or main bronchus measuring 5 × 2 mm were incubated for 90 min at 37°C in 10 ml Hanks’ balanced salt solution buffer (in mM: 5 KCl, 0.3 KH₂PO₄, 138 NaCl, 4 NaHCO₃, and 5.6 Na₂HPO₄) to which 640 units of collagenase (type IV), 10 mg of soybean trypsin inhibitor, and 100 units of elastase (type IV) had been added. The digested tissue was then filtered through a 125-µm Nytex mesh, and the resulting cell suspension was centrifuged. The pellet was then reconstituted in growth medium (Clonetics, San Diego, CA) and plated in 25-cm² flasks. Confluent cells were detached with 0.025% trypsin and 0.02% EDTA and grown on 25-mm-diameter glass coverslips for single-cell imaging of Ca²⁺ transients and contraction studies and on six-well plates for RNA extraction, flow cytometry (fluorescence-activated cell sorting, FACS), and chemotaxis assays. Cells from the 1st to 4th passages were used. They were identified as smooth muscle cells by positive immunohistochemical staining for smooth muscle specific α-actin and positive identification of myosin light chain kinase and calponin by Western blot analysis. This project was approved by the local Institutional Review Boards.

Detection of mRNA Expression of CXCR1 and CXCR2 Using Real-Time PCR

Total RNA was extracted from the cells with TRIZol reagent (GIBCO BRL, Burlington, ON, Canada) according to the manufacturer’s instructions. To analyze messenger RNA (mRNA), we synthesized single-strand cDNA in a 20-µl reaction mixture using 2 µg of total RNA as a template, oligo(dt)₁₂–₁₈ primer, and Superscript II enzyme in the presence of acetylated BSA (GIBCO BRL) and RNA-guard ribonuclease inhibitor (Pharmacia Biotech, Quebec, Canada). PCR reactions were performed (Light Cycler; Roche Molecular Biochemicals) with 20 µl of reaction mixture containing 2 µl of cDNA, 4 µl of both sense and antisense primers for CXCR1 and CXCR2 (CXCR1: sense, 5’-ATGGTCAAAATATTACAGATCC, antisense, 3’-AGATTCATAGACGTTCCCA; CXCR2: sense, 5’-GAGGACCCAGGTGATCCAG, antisense, 3’-GAGGATGTTGAAATGTGCC) or housekeeping gene (β-actin), 1.6 µl of MgCl₂ (3 mM), and 2 µl of fast-start DNA SYBR green with Taq polymerase. The following PCR conditions were used: 45 cycles at 95°C for 10 min of denaturation, followed by PCR at 95°C for 15 s, annealing (CXCR1, 57°C; CXCR2, 58°C, and β-actin, 62°C), and extension at 72°C for 24 s. This reaction was followed by melting curve analysis at parameters of 1 cycle of 95°C followed by 70°C for 30 s and again to 95°C for 20-s transition and ended by cooling.

FACS Analysis

Harvested HASMC (5 × 10⁵ cells/ml) were washed with PBS and then incubated with 0.3% bovine serum albumin and 0.2% naïve rat serum on ice to block nonspecific binding. Fluorescein isothiocyanate- or phycocyanin-conjugated monoclonal antibodies (Abs) to CXCR1 and CXCR2 (BD Biosciences, Mississauga, ON, Canada) or control isotype mouse IgG Ab were incubated with the cells at 4°C for 30 min. After extensive washing, cells were fixed with 1% parafomaldehyde in PBS at 4°C. Cell-associated immunofluorescence was analyzed using a FACScan flow cytometer (BD Biosciences, San Jose, CA) and Cell Quest software to determine the levels of surface expression of CXCR1 and CXCR2 subtypes.

Measurement of Intracellular Ca²⁺

HASMC grown on 25-mm-diameter coverslips were used 10–14 days postplating. Cells were incubated for 30 min at 37°C with Hanks’ buffer (in mM: 137 NaCl, 4.2 NaHCO₃, 10 glucose, 3 Na₂HPO₄, 5.4 KCl, 0.4 KH₂PO₄, 1.3 CaCl₂, 0.5 MgCl₂, 0.8 MgSO₄, and 5 HEPES) containing 5 µM fura-2-acetoxymethyl ester (fura-2 AM) and 0.02% Pluronic F-127 (Molecular Probes, Eugene, OR). The loaded cells were then washed, and the coverslips were placed in a Leiden chamber (Medical Systems, Greenville, NY) containing 450 µl of Hanks’ buffer on the stage of an inverted microscope equipped for cell imaging with a ×40 oil objective (Nikon, Montreal, QC, Canada). The cells were imaged using an intensified camera (Videoscope IC 200) and PTI software (Photon Technology International, Princeton, NJ) at a single emission wavelength (510 nm) with double excitatory wavelengths (345 and 380 nm). The fluorescence ratio (345/380) was measured in randomly selected individual cells (n = 8 per slice), and the free intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) was calculated using a K_d of Ca²⁺ to fura-2 of 224 nM (45). R_max was determined in cells exposed to 10⁻⁵ M ionomycin in the presence of 1.3 mM CaCl₂, and R_min in Ca²⁺-free buffer to which 10⁻³ M EGTA and 10⁻⁵ M ionomycin had been added. Background fluorescence and autofluorescence were automatically subtracted. The contribution of extracellular Ca²⁺ to the peak Ca²⁺ transient was tested by stimulating the cells in Ca²⁺-free Hanks’ buffer containing 1 mM EDTA.

All test drugs were diluted in Hanks’ buffer from frozen stock solutions. They were prewarmed to 37°C before being added in the appropriate concentrations in a 450-µl volume.

Effects of CXCR1 and CXCR2 Neutralizing Antibodies, Inhibition of PLC, and Inositol 1,4,5-Trisphosphate Receptor Inhibition on [Ca²⁺]

HASMC cultured on 25-mm-diameter coverslips were loaded with fura-2 AM for the measurement of [Ca²⁺]. After several washings, the cells were incubated with either CXCR1 or CXCR2 receptor-neutralizing antibodies (20 µg/ml; Research Diagnostic, Flanders, NJ) or vehicle (0.1% DMSO) for 10 min at room temperature, and [Ca²⁺]ᵢ was measured in response to IL-8 (100 nM). For other sets of experiments, the cells were also incubated with either the PLC inhibitor U-73122 (100 µM; Biromol, Plymouth Meeting, PA) or the inactive control U-73345 (100 µM) for 10 min before the addition of IL-8 (100 nM). The effect of inositol 1,4,5-trisphosphate (IP₃) release on [Ca²⁺], was assessed by incubating the cells for 30 min with xestospongin C (10 µM; Biromol) before challenge with IL-8 (100 nM) in Ca²⁺-free medium.

Contraction Studies

HASMC were grown on 25-mm-diameter glass coverslips at an approximate density of 1,500 cells/cm² for 4 days. Images were taken using an inverted microscope with a ×20 magnification objective using Nomarski optics. A charge-coupled device camera (Hamamatsu C 2400) and commercial software were used to acquire the images, which were recorded with ImageMaster (Photon Technology International). To measure the contraction of the cells, images were taken once before and up to 10 min after the addition of IL-8. Images were analyzed with Scion software (National Institutes of Health, Bethesda, MD). The length of the cell was measured before and at different time points (0.5, 1, 2, 5, 7 and 10 min) after the addition of IL-8, and contraction was defined as the percent decrease in cell length from the initial value. All the cells with a well-defined outline present in a given field were measured.

Chemotaxis Assay

Cell migration assays were performed using a modified Boyden chamber (Neuroprobe, Cabin John, MD) (21). Cells were harvested with trypsin (0.025%) and EDTA (0.02%) and were resuspended (8.0 × 10⁵ cells/ml) in serum-free growth medium. A polycarbonate membrane (8.0-µm-pore size) was treated with type I collagen overnight at 4°C and placed between two chambers. The HAMSC were added to the upper chamber, and chemoattractants or vehicle...
(IL-8, PDGF, or PBS in 0.1% BSA) was added to the lower chamber. After 4 h of incubation at 37°C, the membranes were removed, the upper face of the membrane was scraped clear of cells, and the cells that migrated to the lower side of the membrane were fixed and stained with Diff-Quick (Dade Behring, Newark, DE). The number of cells was counted in five random fields under ×40 magnification. Four assays were done in triplicate using cells from three different individuals.

Data Analysis

Data are represented as means ± SE. Comparison of means was performed with paired or unpaired Student's t-tests as appropriate. A difference was considered to be statistically significant when the P value was <0.05.

RESULTS

Expression of mRNA Transcripts for CXCR1 and CXCR2

PCR was performed to determine whether mRNA transcripts for CXCR1 and CXCR2 were expressed in HASMC. PCR products were resolved with agarose gel electrophoresis, and the resulting bands corresponded to the expected base pair sequences (CXCR1, 499 bp; CXCR2, 249 bp). As shown in Fig. 1, HASMC constitutively express mRNA transcripts for both CXCR1 and CXCR2. β-Actin (299 bp) was used as a housekeeping gene.
Surface Expression of CXCR1 and CXCR2

FACS analysis was performed to detect the surface expression of CXCR1 and CXCR2 on HASMC. Five independent experiments were done with cells from three different individuals. Figure 2, A and B, shows that 23.8 ± 2% of cells expressed CXCR1 and 21.2 ± 4% of cells expressed CXCR2, respectively. Human neutrophils were used as positive controls: 99.5% of these cells expressed CXCR1, and 96.2% of the cells expressed CXCR2 (Fig. 2, C and D).

Functionality of IL-8 Receptors in HASMC

Functional activities of CXCR1 and CXCR2 were confirmed by measuring the changes in the [Ca^{2+}]i, and contraction and migration of HASMC cells in response to IL-8.

Effects of IL-8 on [Ca^{2+}]i. [Ca^{2+}]i was measured using single-cell fluorescence imaging, and the Ca^{2+} transients following exposure to IL-8 are shown in Fig. 3A for eight individual cells. The peak Ca^{2+} transient was quite variable from cell to cell, a result consistent with the data presented above showing that the distribution of IL-8 receptors varies from cell to cell. The peak changes in [Ca^{2+}]i were seen between 10 and 20 s after the administration of IL-8 and subsequently returned toward baseline values. The mean increase in Ca^{2+} following increasing concentrations of IL-8 is shown in Fig. 3B. The increase was 16 ± 2 nM (n = 48 cells, 5 different slides) at 10 nM, 54 ± 10 nM (n = 41 cells, 4 different slides) at 100 nM, and 106 ± 17 nM (n = 57 cells, 5 different slides) at 200 nM. The effect of IL-8 on [Ca^{2+}]i was also measured in Ca^{2+}-free medium as shown in Fig. 3C. The absence of extracellular Ca^{2+} did not affect the magnitude of the change in [Ca^{2+}]i, (Ca^{2+}-rich medium, 74 ± 8 nM; Ca^{2+}-free medium, 81 ± 10 nM; not significant) in response to IL-8 (100 nM), indicating that intracellular stores of Ca^{2+} were responsible for the increase in cytosolic Ca^{2+}.

Effects of neutralizing antibodies to CXCR1 and CXCR2 on IL-8-induced increases in [Ca^{2+}]i. HASMC were incubated with either CXCR1- or CXCR2-specific neutralizing antibodies, and [Ca^{2+}]i was measured to determine which of these receptors was involved in Ca^{2+} mobilization. Figure 4, C–F, shows that incubation of cells with either CXCR1 or CXCR2 neutralizing antibodies completely abrogated the changes in [Ca^{2+}]i. After IL-8 stimulation in cells pretreated for 10 min with anti-CXCR1 (20 μg/ml), [Ca^{2+}]i was 104 ± 6 nM, a value comparable to the resting level, and it was 110 ± 3 nM in cells pretreated with anti-CXCR2 (20 μg/ml), whereas the peak Ca^{2+} transient was 246 ± 13 nM in control cells. Histamine-induced Ca^{2+} release was measured after IL-8 stimulation as a positive control to confirm the capacity of the cells to respond.

Effects of PLC inhibitor U-73122 and IP3 receptor blocker xestospongin C on IL-8-induced [Ca^{2+}]i. To determine the role of PLC in mediating the IL-8-induced changes in [Ca^{2+}]i, we incubated cells with either the PLC inhibitor U-73122 (10 μM; n = 38 cells) or its inactive analog, U-73343 (100 μM; n = 38 cells) for 10 min, and [Ca^{2+}]i was measured after the addition of IL-8 (100 nM). U-73122 completely blocked the IL-8-induced changes in [Ca^{2+}]i, whereas the control compound U-73343 did not have any effect. To further confirm that Ca^{2+} release was the result of PLC activation and the subsequent release of IP3, we assessed the effect of xestospongin C, a cell-permeable inhibitor of IP3 receptor-mediated Ca^{2+} release, in Ca^{2+}-free medium. Pretreatment of the cells with xestospongin C (10 μM; 30 min; n = 48 cells) abolished IL-8-induced peak Ca^{2+} (98 ± 7 vs. 121 ± 7 nM in vehicle-incubated cells; P = 0.026).

Effects of IL-8 on HASMC contraction. IL-8-induced contraction of HASMC was measured by stimulating cells with IL-8 (100 nM) or vehicle. Figure 6, A and B, shows the changes in cell length 10 min after addition of IL-8. Contraction was assessed by measuring all the cells clearly delineated on a given slide. Cells from six different individuals (8–20 cells/
slide) were analyzed. The addition of IL-8 significantly decreased the length of the cells by 14.5 ± 0.1% (n = 348 cells) compared with the vehicle-treated cells, which decreased in length by 4.8 ± 1.3% (n = 104 cells). The half-time for changes in length was calculated to be 2 min, approximately. The contribution of CXCR1 vs. CXCR2 receptor subtype to the IL-8-induced contraction was assessed by measuring IL-8-induced changes in cell length after 10-min preincubation of the cells with CXCR1 and CXCR2 neutralizing antibodies (20 μg/ml). The results are shown in Fig. 6 as cumulative frequency curves. Each symbol represents one cell. There was a significant difference between PBS- and IL-8-treated cells (P = 0.0005; Kolmogorov-Smirnov test). Furthermore, CXCR1 and CXCR2 neutralizing antibodies significantly inhibited the effects of IL-8 (CXCR1, P = 0.001; CXCR2, P = 0.015).

**Effects of IL-8 on HASMC migration.** Chemotaxis assays were performed to determine whether IL-8 induced migration of HASMC. Figure 7 shows that IL-8 exposure (10 and 100 nM) induced a 2.3-fold increase in cell migration at a concentration of 100 nM and a 1.5-fold increase at 10 nM (n = 4 separate experiments with 4 different subjects). PDGF (5
ng/ml) caused a 2.8-fold increase in cell migration and served as a positive control (data not shown). To determine the role of the subtypes of IL-8 receptor, we assessed migration after pretreatment with CXCR1 and CXCR2 neutralizing antibodies. The results show that migration was abolished after pretreatment with either subtype of receptor neutralizing antibodies (n = 3 different experiments with 3 different subjects).

**DISCUSSION**

IL-8 (CXCL8) is a chemokine released by various cell types, including monocytes and macrophages. It has been implicated in various types of inflammatory reactions, including myocardial infarct, rheumatoid arthritis, and asthma (38). In addition to its potent chemotactic properties, IL-8 is a proangiogenic mediator and also possesses tumorigenic properties, making it a prime candidate for anticytokine therapy (7). In the lungs, macrophage- and epithelial cell-derived IL-8 induces airway neutrophilic inflammation and thus could be of importance in certain forms of asthma and other airway diseases such as COPD and CF. Increased levels of IL-8 have been measured in the sputum, bronchoalveolar lavage, and sera of patients with CF and COPD (29), and it also has been reported that IL-8 expression is increased in the bronchial epithelium of asthmatic patients (13, 30). These increased levels likely contribute to the airway neutrophilia associated with these diseases (39). Indeed, monoclonal anti-IL-8 antibody therapy has been proposed for the treatment of COPD, and pilot clinical trials have been conducted (29).

IL-8 acts through the specific receptors CXCR1 and CXCR2 on target cells. In neutrophils, it causes changes in [Ca\(^{2+}\)], degranulation, and chemotaxis (5, 22, 33). Other cell types, including monocytes (42), endothelial and bronchial epithelial cells, as well as neurons and tumor cells (11, 16, 46–48), also express CXCR1 and CXCR2. Although HASMC have been shown to synthesize IL-8 in response to the proinflammatory mediators TNF-α, IL-1-β (23), and bradykinin (35), it was not known whether these cells constitutively express CXCR1

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**Fig. 5.** Effects of PLC inhibitor (U-73343), inactive PLC inhibitor (U-73122), and inositol 1,4,5-trisphosphate (IP\(_3\)) receptor inhibitor (xestospongin C) on peak [Ca\(^{2+}\)]. *P < 0.05; **P < 0.03. Data presented are means ± SE.

**Fig. 6.** Contraction of HASMC with IL-8. Contraction of the cells with IL-8 (100 nM) was detected using phase-contrast microscopy. A and B: representative examples of images captured before (A) and 10 min after (B) the addition of IL-8. Numbers 1–3 indicate cells that contracted with IL-8 treatment. C: percent decrease in the cell length after treatment with IL-8 and PBS and after preincubation with neutralizing antibodies to receptors CXCR1 and CXCR2. Each symbol represents 1 cell.
and/or CXCR2 and therefore could also react to IL-8. In the current study, we demonstrated that HASMC constitutively express mRNA and protein for both CXCR1 and CXCR2 receptors. The receptors are functional, and stimulation causes changes in \( [\text{Ca}^{2+}]_i \), contraction, and migration of HASMC.

CXCR1 and CXCR2 are G protein-coupled receptors and activate PLC and inositol phosphate hydrolysis, leading to the mobilization of intracellular \( \text{Ca}^{2+} \) (28). Both receptors were expressed to a comparable extent; flow cytometry showed that 23% of the cells expressed CXCR1 and 21% of the cells expressed CXCR2. However, the level of expression of both receptors is relatively low compared with neutrophils, 98% of which express the receptors. Intracellular \( \text{Ca}^{2+} \) was released within HASMC in response to IL-8 in a manner that was quite variable from cell to cell. The proportion of cells that demonstrated \( \text{Ca}^{2+} \) transients appeared to be higher than the number contracting, suggesting different thresholds for these events. It is also possible that once activated, cells might release other mediators (adenosine triphosphate, for example), thereby triggering \( \text{Ca}^{2+} \) responses in adjacent cells. The \( \text{Ca}^{2+} \) transients were unaffected by removal of \( \text{Ca}^{2+} \) from the extracellular medium, indicating that IL-8 released \( \text{Ca}^{2+} \) from intracellular sources. This observation was confirmed by the fact that \( \text{Ca}^{2+} \) release was blocked by U-73122, a PLC inhibitor, and by xestosponglin C, an inhibitor of IP\( _3 \)-induced \( \text{Ca}^{2+} \) release. These data are consistent with the results obtained with human neutrophils (10), showing that IL-8 induces an increase in \( [\text{Ca}^{2+}]_i \) through release from intracellular sources and not from influx from the extracellular milieu.

Exposure to IL-8 caused HASM cell contraction. This effect was specific, because it was blocked by receptor neutralizing antibodies of either receptor subtype. A bronchoconstrictive effect of IL-8 has been shown in vivo in guinea pigs, although this effect was due in part to the release of histamine by mast cells (17), and it also has been reported that IL-8 increases acetylcholine-induced contraction in rat intestinal segments (37). However, a direct effect of IL-8 on smooth muscle contraction could not be inferred from these experiments. Thus our data show that IL-8 has a direct stimulatory effect on smooth muscle cells. Not all the cells contracted, which is consistent with the low level of expression of the receptors. Nevertheless, the results suggest that IL-8 release by neutrophils, airway epithelial cells, fibroblasts, mast cells, and macrophages present in the airways could cause bronchoconstriction through a direct effect.

IL-8 is a potent chemotactic agent for neutrophils monocytes and eosinophils, but it also has been shown to cause migration of rat aortic smooth muscle cells (52, 53). In asthma and cystic fibrosis, it has been postulated that migration of ASM cells may be one of the mechanisms involved in the airway remodeling often observed in these diseases (24, 32). Parameswaran et al. (36) have shown that ASM cells show a chemotactic response to PDGF and that this response is augmented by cysteinyl leukotrienes, mediators that have been shown to be important for airway remodeling in animal models of allergic asthma. Our results show that IL-8 increases the migration of HASMC 2.5-fold, a chemotactic effect comparable to that induced by PDGF. The increase in cell migration induced by IL-8 was lower than that observed in neutrophils (12, 36) but of the same order of magnitude as that observed for vascular smooth muscle cells (51) with PDGF stimulation. Whether IL-8 secreted in vivo is in sufficiently high concentrations to produce the effects described in HASM cells is not known. Of interest, however, is the fact that IL-8 has a very long half-life and can remain at the site of inflammation for several days (38). It is thus conceivable that continuous secretion of IL-8 could lead to accumulation of the chemokine in concentrations high enough to stimulate smooth muscle.

Selective neutralizing of CXCR1 and CXCR2 with antibodies abrogated the release of \( \text{Ca}^{2+} \), contraction, and chemotaxis, confirming that both the CXCR1 and CXCR2 receptors could mediate these effects. The abrogation of signals that we observed with the receptor neutralization experiments suggests that coordinated effects or differential interaction of both subtypes of receptors are necessary for \( \text{Ca}^{2+} \) release and that \( \text{Ca}^{2+} \) release is necessary for contraction and chemotaxis to occur in response to IL-8. The complete lack of response to IL-8 after either receptor blockade could also occur if CXCR1 and CXCR2 formed heterodimers. It was initially reported by Trettel et al. (44) that CXCR2 receptors form dimers in HEK-293 cells and in cerebellar neurons but that CXCR1 does not. However, Wilson et al. (48a) have recently demonstrated that CXCR1 and CXCR2, when coexpressed in HEK-293 cells, form heterodimers. Through an elegant series of studies with single-cell imaging of fluorescence resonance energy transfer, cell surface time-resolved fluorescence resonance energy transfer, and bioluminescence resonance energy transfer, the authors showed that these interactions between CXCR1 and CXCR2 occur during protein synthesis, before translocation of the receptors to the cell surface. They also showed that the heterodimers have equal apparent affinities for the ligand. The results we report are consistent with their observations and suggest that such a phenomenon could occur in HASMC.
In conclusion, we have demonstrated that IL-8 induces Ca^{2+} mobilization, contraction, and migration of HASMC. These cells express both CXCR1 and CXCR2 mRNA transcripts and surface receptor proteins. Both receptor subtypes appear to be important in mediating the effects of IL-8 on the cells. On the basis of these findings, we speculate that IL-8 may contribute to airway narrowing, airway hyperresponsiveness, and airway remodeling in airway diseases such as asthma, CF, and COPD by mechanisms that are independent of the inflammatory response that it also engenders.

GRANTS

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REFERENCES


10. C964 IL-8 INDUCED CONTRACTION IN HUMAN AIRWAY SMOOTH MUSCLE


