IL-13 and IL-4 Up-Regulate Cysteinyloleukotriene 1 Receptor Expression in Human Monocytes and Macrophages

Maryse Thivierge, Jana Stanková and Marek Rola-Pleszczynski

*J Immunol* 2001; 167:2855-2860; doi: 10.4049/jimmunol.167.5.2855

http://www.jimmunol.org/content/167/5/2855

References

This article cites 35 articles, 18 of which you can access for free at:

http://www.jimmunol.org/content/167/5/2855.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
IL-13 and IL-4 Up-Regulate Cysteinyl Leukotriene 1 Receptor Expression in Human Monocytes and Macrophages

Maryse Thivierge, Jana Staňková, and Marek Rola-Pleszczynski

The cysteinyl (Cys) leukotrienes (LT)C₄, LTD₄, and LTE₄, are lipid mediators that have been implicated in the pathogenesis of asthma. The human LTD₄ receptor (CysLT₁R) was recently cloned and characterized. The present work was undertaken to study the potential modulation of CysLT₁R expression by the Th2 cytokines IL-13 and IL-4. In this study, we report that IL-13 up-regulates CysLT₁R mRNA levels, with consequently enhanced CysLT₁R protein expression and function in human monocytes and monocyte-derived macrophages. CysLT₁R mRNA expression was augmented 2- to 5-fold following treatment with IL-13 and was due to enhanced transcriptional activity. The effect was observed after 4 h, was maximal by 8 h, and maintained at 24 h. IL-4, but not IFN-γ, induced a similar pattern of CysLT₁R up-regulation. Monocytes pretreated with IL-13 or IL-4 for 24 h showed enhanced CysLT₁R protein expression, as assessed by flow cytometry using a polyclonal anti-CysLT₁R Ab. They also showed enhanced responsiveness to LTD₄, but not to LTB₄, in terms of Ca²⁺ mobilization, as well as augmented chemotactic activity. Our findings suggest a possible mechanism by which IL-13 and IL-4 can modulate CysLT₁R expression on monocytes and macrophages, and consequently their responsiveness to LTD₄, and thus contribute to the pathogenesis of asthma and allergic diseases. The Journal of Immunology, 2001, 167: 2855–2860.

L

euokotrienes (LTs),³ which are derived through the 5-lipoxygenase pathway of arachidonic acid metabolism, are lipid mediators of inflammation and immediate hypersensitivity (1–3). The cysteinyl (Cys)LTs, LTC₄, LTD₄, and LTE₄ (components of the slow-reacting substance of anaphylaxis), are potent lipid mediators implicated mainly in acute bronchoconstriction and chronic airway inflammation in asthma. When instilled into the airways, they mimic many of the features of human asthma, including bronchoconstriction, mucus secretion, and airway hyperresponsiveness (AHR) (1–4). Recently, Lynch et al. (5) and Sarau et al. (6) reported the cloning and characterization of a high affinity cell surface human LTD₄ receptor (CysLT₁R) that belongs to the G protein-coupled receptor family. In normal lung, CysLT₁R mRNA was found to be expressed in macrophages as well as in peribronchial smooth muscle (5).

The inflammatory pathology associated with asthma is thought to be mediated by Th2 lymphocytes and their cytokine network. Numerous studies of bronchoalveolar lavage (BAL) and biopsies from asthmatic airways have shown an increase in CD4⁺ T lymphocytes producing Th2-like cytokines, IL-4, IL-5, and IL-13 (7–9). They have suggested the importance of these cytokines in AHR, which is one of the defining features of asthma and believed to result from chronic inflammation of the bronchial mucosa. Significantly elevated expression of IL-13 mRNA and protein has been observed in BAL cells of patients with atopic asthma after allergen challenge (10). More recently, blockade of IL-13 before aeroallergen challenge was shown to be sufficient to attenuate AHR (11). Unlike IL-4, the production of IL-13 can be sustained through the late asthmatic response, and the concentration of secreted IL-13 strongly correlates with the number of eosinophils in BAL and in bronchial submucosa (12). Together, these results suggest that IL-13 plays an important role in the pathogenesis of asthma.

IL-13 and IL-4 have several overlapping biological activities (13, 14) that may be due to a shared receptor subunit, IL-4Rα (15), and signaling through a shared STAT6-dependent pathway (16). In addition to potent anti-inflammatory properties on macrophages and other cells (17–20), both cytokines also induce several immunostimulatory functions, such as increase in VCAM-1 expression on the surface of endothelial cells and induction of monocyte chemoattractant protein-1 (MCP-1) production (21, 22). Because both LTs and Th2 cytokines are major players in asthma and because the level of expression of CysLT₁R could be expected to affect cellular responses to LTD₄, we initiated the present study to investigate the potential for IL-13 and IL-4 to modulate the expression and function of CysLT₁R.

Materials and Methods

Cells

Monocytes were obtained from peripheral blood of healthy medication-free volunteers, following informed consent in accordance with an Internal Review Board-approved protocol. Peripheral blood leukocytes were enriched by dextran sedimentation, layered over a Ficoll-Hypaque cushion, and centrifuged at 400 × g for 20 min. Mononuclear leukocytes were collected at the interface and washed twice with PBS and resuspended in RPMI 1640 (Life Technologies, Burlington, Ontario, Canada) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich, Oakville, Ontario, Canada), 100 U/ml penicillin, and 100 µg/ml streptomycin in a humidified atmosphere with 5% carbon dioxide at 37°C. Monocytes were then purified by adherence (60 min, 37°C) on plastic petri dishes coated with defibrinized autologous serum and removed with EDTA (0.01 M) in RPMI 1640–10% FBS. Cells were resuspended in RPMI 1640–10% FBS at 1 × 10⁵ cells/ml and allowed to rest overnight before stimulation with the appropriate stimuli.
Adherent cells cultured on plastic petri dishes for up to 5 days were referred to as monocyte-derived macrophages.

Cytokines and reagents

Human rIL-4 and rIL-13 were obtained from PeproTech (Rocky Hill, NC); human IFN-γ was obtained from R&D Systems (Minneapolis, MN); all cytokine preparations contained <0.1 ng endotoxin per microgram (1 EU/μg); rabbit polyclonal anti-human CysLT1 R Ab was developed and characterized with Cayman Chemical (Ann Arbor, MI); rabbit IgG isotype control Ab was obtained from Biozol (Eberbach, Germany; Catalogue No. 38913), and platelet-activating factor (PAF) were obtained from Cayman Chemical; MK571 was obtained from Biomol (Plymouth Meeting, PA).

Northern blot analysis

Total cellular RNA was extracted by the guanidinium thiocyanate method (23), separated by electrophoresis on 1% agarose, and transferred onto a Hybond-N+ membrane (Amersham Pharmacia Biotech, Baie d’Urfe, Quebec, Canada) for Northern analysis. The cDNA corresponding to the whole coding sequence of human CysLT1 R (5) was amplified by PCR from cDNA of human monocytes, using the primers 5′-CGGATCCGGATCA AACAGGAAATC-3′ as sense and 5′-CCGGATCTCAATGGTTTAGA AATC-3′ as antisense. The amplified CysLT1 R fragment contained 1014 bp. Control hybridizations were performed with the human GAPDH (49) cDNA probe obtained from the American Type Culture Collection (Manassas, VA). The probe was labeled with a multiprime DNA labeling system (Amersham Pharmacia Biotech) using [α-32P]dCTP (sp. act., 3000 Ci/mmole; Amersham Pharmacia Biotech). Membranes were prehybridized for 4 h in a mixture containing 120 mM Tris (pH 7.4), 600 mM NaCl, 8 mM EDTA (pH 8), 0.1% sodium pyrophosphate, 0.2% SDS, and 100 μg/ml heparin; hybridization was performed overnight at 60°C in the same mixture in which the concentration of heparin was increased to 625 μg/ml and dextran sulfate at 10% was added. The membranes were then washed once at room temperature for 20 min in 2× SSC (1× SSC: 0.15 M NaCl, 0.15 M sodium citrate (pH 7)) and once with 0.1× SSC. The membranes were exposed to Hyperfilm MP (Amersham Pharmacia Biotech) with intensifying screens at −80°C.

Nuclear run-on transcription assay

Before nuclei isolation, monocytes were stimulated with 3 or 6 ng/ml of IL-13 (10 ng/ml). Following stimulation, cells were washed with ice-cold PBS, and pelleted at 250 × g for 5 min. The cell pellet (5 × 107 cells) was then resuspended in 1 ml lysis buffer (10 mM Tris-HCl (pH 7.4), 10 mM KCl, 5 mM MgCl2, 0.5% Nonidet P-40 (v/v)), incubated for 5 min on ice, and centrifuged at 500 × g for 5 min at 4°C. The supernatant was discarded, and the nuclei were resuspended in 200 μl freezing buffer (50 mM Tris- HCl, 5 mM MgCl2, 1 mM EDTA) and quickly frozen in liquid nitrogen. For the transcription assay, nuclei were thawed on ice and pelleted at 500 × g for 30 s at 4°C, and the supernatant was discarded. Nuclei were then mixed with 100 μl reaction buffer containing 100 mM Tris-HCl (pH 7.9), 300 mM (NH4)2SO4, 4 mM MgCl2, 200 mM NaCl, 0.4 mM EDTA, 1 mM DTT, 40% glycerol, 1 mM each of ATP, CTP, and GTP, 1 μl RNAse inhibitor (40 U/μl RNAse; Promega, Madison, WI), and 150 μCi [α-32P]UTP (3000 Ci/mmole; Amersham Pharmacia Biotech) and incubated at 30°C for 30 min. The 32P-labeled RNA transcripts were then isolated with saturated phenol solution and chloroform-isooamyl alcohol (49/1), mixed well, incubated on ice for 15 min, and then centrifuged for 30 min at 500 × g at 4°C. The elongated 32P-labeled RNA was then purified on a Sephadex G-25 column. Before ethanol precipitation, 32P-labeled RNA transcripts were then isolated with saturated phenol solution and chloroform-isooamyl alcohol (49/1), mixed well, incubated on ice for 15 min, and then centrifuged for 15 min at 500 × g at 4°C. The elongated 32P-labeled RNA was then purified on a Sephadex G-25 column. Before ethanol precipitation, 32P-labeled RNA transcripts were denatured adding NaOH (final concentration of 0.2 M) for 15 min on ice. The solution was neutralized by the addition of HEPES, pH 5.5, 10 mM EDTA, 1% SDS, and 50 mM NaCl, and incubated for 20 min at 37°C. Elongated 32P-labeled RNA transcripts were then precipitated adding 1 vol of ethanol and 0.1 vol of 3 M sodium acetate and centrifuged at 15 min at 500 × g at 4°C. The pellet was resuspended in 500 μl hybridization solution (0.75% NaCl, 50 mM HEPES (pH 7), 2 mM EDTA (pH 8), 50% deionized formamide, 0.5× SDS, 10× Denhardt’s, and 0.5 mg/ml salmon sperm DNA). RNA was then denatured at 90°C for 5 min and hybridized at 42°C for 48 h to 5 μg denatured DNA immobilized on positively charged nylon transfer membrane (Mandel, Saint-Laurent, Quebec, Canada) in 3 ml hybridization solution. Membranes were then washed four times at 42°C for 15 min in 0.1× SSC, 0.1% SDS and exposed to Hyperfilm MP with intensifying screens at −80°C for 2 wk. For immobilization of DNA to membranes, 5 μg cDNA was denatured with 0.3 M NaOH at room temperature for 30 min, neutralized with ammonium acetate (final concentration of 1.5 M), and spotted onto nylon membrane using a slot blot apparatus.

Flow cytometry

For flow cytometry studies, cells were washed with PBS and fixed with 2% paraformaldehyde for 15 min at room temperature, followed by permeabilization with 0.1% saponin for an additional 15 min at room temperature. Cells were resuspended with PBS-2% BSA and labeled for 30 min at 4°C with anti-CysLT1 R Ab or with isotype control Ab. Cells were then washed twice in PBS and incubated for 30 min at 4°C with FITC-conjugated goat-antirabbit IgG. Finally, cells were washed again and resuspended in PBS before single-color immunofluorescence analysis of 5000 cells was performed on a FACSscan flow cytometer (BD Biosciences, San Jose, CA).

Intracellular calcium mobilization

For Ca2+ mobilization assays, 5 × 104 cells were loaded in HBSS (Life Technologies) containing 350 μg/ml NaHCO3 and 10 mM HEPES (pH 7) with the calcium indicator fura 2-AM (Molecular Probes, Eugene, OR) for 30 min at room temperature. Loaded cells were washed twice, suspended in fresh loading buffer, and added to a constantly stirred cuvette, maintained at 37°C in a SLM/Aminco spectrophotometer (SLM Instruments, Urbana, IL). The concentration of extracellular calcium was brought to 1.5 mM by addition of a solution of CaCl2 into the cuvette 10 min before recordings. Maximal cell fluorescence (Fmax) was obtained by adding Triton X-100 to a final concentration of 0.5%. Minimal fluorescence (Fmin) was determined by subsequent addition of the chelator EGTA in Tris-HCl buffer (100 mM (pH 9)) at 125 mM. Stimuli consisted of LTD4, LTB4, and PAF.

Chromatase assay

Monocytic chromatase activity was performed with Boyden chambers using a modified Boyden chamber chromatase assay. A volume of 200 μl cells (6 × 105) in RPMI 1640, supplemented with 2.5 mg/ml BSA, was added to the upper chamber. The lower chamber contained graded concentrations of LTD4 or its vehicle. For chemokinesis studies, both chambers contained LTD4 or its vehicle. The two chambers were separated by a 5-μm-pore-size polycarbonate filter (Osmonics, Westborough, MA). After incubation for 2 h at 37°C in 5% CO2, the chambers were disassembled and the upper side of the filter was scraped free of cells. Cells on the lower side were removed with 5 mM EDTA, centrifuged, and resuspended in PBS. An aliquot of 100 μl was then counted in the FACSscan.

Results

The expression of CysLT1 R in human monocytes was first investigated by Northern blot analysis of total RNA following 8 h of stimulation with graded concentrations of IL-13 ranging from 1 to 20 ng/ml. As shown in Fig. 1A, human monocytes constitutively expressed low levels of CysLT1 R mRNA, and IL-13 induced a time-dependent augmentation of transcript levels. Accumulation was augmented 3- to 5-fold over baseline, and the effect was detectable as soon as 4 h of stimulation, was maximal at 8 h, and was still detectable at 24 h. IL-13 induced a concentration-dependent augmentation in steady state levels of CysLT1 R mRNA, which plateaued at 10 ng/ml IL-4 (Fig. 1B). In parallel experiments, we also examined the effect of the cytokine IL-4 on CysLT1 R mRNA expression. Fig. 1C illustrates CysLT1 R mRNA levels in monocytes and monocyte-derived macrophages following 6 h of incubation in the absence or presence of IL-13 (10 ng/ml) or IL-4 (10 ng/ml). Both cytokines induced similar levels of CysLT1 R mRNA expression in both cell populations. Fig. 1D illustrates the kinetics of up-regulation of CysLT1 R mRNA expression by IL-4, with a maximal effect at 4 h of incubation. Fig. 1E shows the effect to be concentration dependent, with up-regulation detectable at 0.1 ng/ml IL-4. Moreover, the Th1-type cytokine IFN-γ was not capable of up-regulating CysLT1 R.

In a further series of experiments, we evaluated the mechanisms underlying the accumulation of CysLT1 R mRNA induced by IL-13. The augmentation of CysLT1 R mRNA accumulation in monocytes following IL-13 treatment could result from augmentation in mRNA
stability and/or gene transcription rate. We first examined the former possibility by assessing CysLT₁R mRNA decay kinetics with medium- and IL-13-treated monocytes using the RNA synthesis inhibitor, actinomycin D (10 μg/ml). Monocytes were incubated 4 h in the presence or absence of IL-13 before addition of actinomycin D, and CysLT₁R mRNA levels were assessed by Northern blot analysis after periods of 0–180 min. As illustrated in Fig. 2A, CysLT₁R mRNA t_{1/2} was ~2.5 h in unstimulated human monocytes. IL-13 treatment did not induce a significant change in CysLT₁R mRNA t_{1/2}, suggesting that the increased accumulation of CysLT₁R mRNA by IL-13 was not caused by stabilization of the transcripts. To assess whether IL-13 induced CysLT₁R mRNA accumulation through a transcriptional mechanism, we performed experiments in which monocytes were pretreated with actinomycin D for 15 min to block new RNA synthesis and were then treated with either medium or IL-13. As shown in Fig. 2B, this pretreatment completely abolished the IL-13-induced accumulation of CysLT₁R mRNA, suggesting a transcriptional mechanism in the regulation of CysLT₁R expression by IL-13. Transcriptional activation of the CysLT₁R gene was confirmed by a nuclear run-on experiment (Fig. 2C) that showed that nuclear transcription of the CysLT₁R gene was indeed increased (4-fold at 3 h) by IL-13 treatment.

The next series of experiments examined whether transcription of CysLT₁R mRNA was associated with an augmented cellular expression of CysLT₁R protein. Changes in monocyte mRNA levels were paralleled by an increase in cellular expression of CysLT₁R, as assessed by flow cytometry using specific anti-CysLT₁R Ab. As illustrated in Fig. 3A, both IL-13 and IL-4 induced a markedly augmented expression of CysLT₁R protein in monocytes. The effect was maximal following a 24-h stimulation with the cytokines. The effects of the two cytokines were both concentration and time dependent, being maximal at 10 ng/ml after 24 h (Fig. 3, A and C). Similarly to monocytes, IL-13 and IL-4 also up-regulated the expression of CysLT₁R in monocyte-derived macrophages (Fig. 3A).

We finally investigated whether the IL-13- and IL-4-induced up-regulation of CysLT₁R expression was associated with augmented functional responsiveness to LTD₄. Receptor function was evaluated by mobilization of intracellular calcium upon stimulation with LTD₄. As illustrated in Fig. 4A, monocytes cultured for 24 h with 10 ng/ml IL-13 or IL-4 showed increased calcium flux in response to LTD₄ (Δ intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) = 129.5 ± 34.9 nM for control cells vs 251.8 ± 63.7 nM for IL-13-treated cells (p < 0.02), and 244 ± 66.8 nM for IL-4-treated cells (p < 0.05), n = 4), but not to LTB₄ (Δ[Ca²⁺]ᵢ = 111.8 ± 25.5 nM for control cells vs 132.8 ± 38.2 nM for IL-13-treated cells (p > 0.1), and 149.4 ± 28.4 nM for IL-4-treated cells (p > 0.1), n = 5), nor to PAF (data not shown). The response to LTD₄a, but not to LTB₄, was totally blocked by the selective CysLT₁R antagonist MK571. As shown in Fig. 4B, responsiveness to LTD₄ in monocyte-derived macrophages was augmented even more than in monocytes, following a 24-h incubation with the cytokines IL-13 or IL-4 (10 ng/ml).

The functional relevance of increased CysLT₁R expression on IL-13-stimulated monocytes was also investigated in terms of modulation of their chemotactic response to LTD₄a. Whereas resting monocytes showed a weak chemotactic response to LTD₄a, Fig. 5A shows that a significantly augmented response to LTD₄a was observed when monocytes had been exposed for 24 h to IL-13. Enhanced migration to LTD₄a was concentration dependent, with activity already present at 1 nM and reaching a plateau between 10 and 100 nM LTD₄a. Similar results were obtained with IL-4-treated cells. Chemokinesis was not affected, however, by pretreatment of cells with either IL-4 or IL-13 (Fig. 5B). Moreover, monocyte chemotactic responses to the chemokine MCP-1 were not affected by such pretreatment (Fig. 5B). These observations suggest that the augmentation of CysLT₁R expression following stimulation with IL-13 or IL-4 is accompanied by an enhanced functional activity of the receptor.

Discussion

The CysLTs, particularly LTD₄ and LTC₄, are lipid mediators that have been implicated in the pathogenesis of several inflammatory processes, including asthma. Their multiple and diverse cellular actions make them also potential protagonists in a number of other pathological states, such as adult respiratory distress syndrome and pulmonary hypertension. The present work was initiated to study the mechanisms by which the receptor for LTD₄ can be regulated. Our understanding of the mechanisms governing the regulation of CysLT₁R expression is a prerequisite for attempting to modulate and control CysLT₁R expression.
expression in various tissues in LTD₄-mediated diseases, and particularly asthma. In the present study, we have shown that IL-13 and IL-4, two prototypic Th2 cytokines that share many biological effects, up-regulate the expression of the CysLT₁ R in human monocytes and monocyte-derived macrophages. This up-regulation involves transcriptional activation of the CysLT₁ R gene and is associated with enhanced CysLT₁ R protein expression and augmented responsiveness to the ligand LTD₄ in terms of intracellular calcium mobilization and chemotactic activity. In contrast, the prototypic Th1 cytokine IFN-γ did not modify CysLT₁ R expression.

The pathology associated with asthma is thought to be mediated by Th2 cells and involves potentially important roles for the cytokines IL-4, IL-13, and IL-5, because both mRNA and protein levels of these cytokines are elevated in allergic patients as compared with normal individuals (7–10, 24). A particular association between IL-13 and asthma has also been suggested by several studies. IL-13 was produced by BAL cells of atopic asthma patients after allergen challenge (7), and increased IL-13 mRNA was detected in the bronchial mucosa of asthmatic patients (10). More recently, several studies have demonstrated the central role IL-13 appears to play, at least in experimental asthma, in terms of induction of AHR and mucus hypersecretion (11, 25, 26). Its preferential role in asthma may be due, in part, to its longer half-life in vivo and its higher levels in asthmatic lung, compared with IL-4, and, in part, to its predominant effect on some features of asthma, such as goblet cell activation (11, 25).

Of the different mediators that are known to be involved in asthma, LTs are considered to be among the most important because they participate in both the bronchoconstriction and the inflammatory components of the disease. In general, cyclooxygenase metabolites of arachidonate are associated with nonallergic inflammation, whereas 5-lipoxygenase metabolites such as LTB₄, LTC₄, and LTD₄ are predominantly involved in allergic inflammation. In this context, it is quite interesting that the allergy-associated cytokines IL-13 and IL-4 tend to suppress cyclooxygenase-2 activity (17, 27), whereas they enhance LTA₄ hydrolase activity, resulting in the increased production of LTD₄ (28). In other reports, IL-13 was shown to enhance

![FIGURE 2](image-url)

**FIGURE 2.** Transcriptional regulation of CysLT₁ R receptor gene expression following stimulation with IL-13. A. Effect of IL-13 (10 ng/ml) on the CysLT₁ R mRNA stability. Monocytes were pretreated for 4 h with IL-13 before addition of actinomycin D, and CysLT₁ R mRNA half-life was measured and expressed as percentage of values at time 0. B. Monocytes were pretreated for 15 min with either medium or actinomycin D (10 μg/ml) before stimulation with IL-13 (10 ng/ml) for 3 h. Total RNA was extracted and used in Northern blot analysis, as described in Materials and Methods. C. Nuclear run-on assay. Monocytes incubated in the absence or presence of 10 ng/ml IL-13 for 3 or 6 h. Equal loading and transfer were assessed by comparison with GAPDH gene expression.

![FIGURE 3](image-url)

**FIGURE 3.** Flow cytometric analysis of CysLT₁ R expression in cells stimulated with IL-13 or IL-4. Human monocytes or monocyte-derived macrophages were incubated with IL-13 or IL-4 (10 ng/ml) for 24 h. Cells were subsequently labeled with anti-CysLT₁ R or isotype-matched control Abs, followed by incubation with FITC-conjugated goat anti-rabbit IgG. A. Results of a single experiment, representative of at least four, are shown. Dotted lines represent labeling with isotype control Ab. Solid thin and thick lines represent labeling of medium- and cytokine-treated cells, respectively, with anti-CysLT₁ R Ab. Concentration-dependent (B) and time-dependent (C) effects are presented as mean channel fluorescence values (n = 4–6; * p < 0.05; ** p < 0.01 vs untreated cells).
15-hydroxyeicosatetraenoic acid release in monocytes (19, 29), to increase cytosolic phospholipase A2 expression, and to modulate zymosan-stimulated arachidonic acid mobilization (30).

Excessive production of leukocytes and their subsequent invasion of the airways and other target organs are characteristic features of asthma and allergic diseases. Thus, priming of leukocytes with cytokines such as IL-13 and IL-4, combined with an increase in CysLT production in the airways of asthmatics, may contribute to the influx of airway inflammation.

**FIGURE 4.** [Ca^{2+}]_i was determined using the fluorescent dye fura 2-AM. A. Human monocytes were cultured for 24 h in the absence or presence of IL-13 or IL-4 (10 ng/ml), loaded with fura 2-AM, and stimulated with 100 nM LTD_4 or 100 nM LTB_4, in the absence or presence of the CysLT,R antagonist MK571 (1 μM). B. Human monocyte-derived macrophages were cultured for 24 h in the absence or presence of IL-13 or IL-4 (10 ng/ml), loaded with fura 2-AM, and stimulated with 100 nM LTD_4.

**FIGURE 5.** Effect of IL-13 and IL-4 on chemotactic response of monocytes to LTD_4. A. Monocytes were incubated in the absence or presence of 10 ng/ml IL-13 or IL-4 for 24 h and then assessed for their ability to migrate across a 5-μm-pore-size polycarbonate filter in response to graded concentrations of LTD_4. Results are from three independent experiments. *, p < 0.05; **, p < 0.01 vs untreated cells. B. Comparative responses of monocytes to LTD_4 (100 nM) and MCP-1 (50 ng/ml) following incubation for 24 h in the presence or absence of 10 ng/ml IL-13 or IL-4. Chemokinesis was also assessed in the presence of vehicle (M/M) or LTD_4 (D4/D4) in both chambers and was not affected by pretreatment with the cytokines.
and activation of leukocytes. In another receptor system, IL-13 and IL-4 were shown to augment the expression of the chemokine receptors CXCR1 and CXCR2 in human monocytes (31). Hence, our findings, in addition to the latter observations, suggest that, in Th2-dominated responses, IL-13 and IL-4 could participate in the recruitment and activation of mononuclear phagocytes. We have also recently shown that CysLT1R expression can be up-regulated by the Th2 cytokine IL-5 in eosinophil-differentiated HL-60 cells (32).

IL-13 exhibits pleiotropic biological functions on multiple cell types, and it shares one chain of its receptor with IL-4. Whereas Janus kinase (JAK)3 is one of the kinases that transduces signals from the 4R (33), the signaling pathway of IL-13 seems to be quite variable depending on the cell type. Tyrosine kinases were recently suggested to play a role in the signaling pathway following binding of IL-13, which was shown to induce JAK3 phosphorylation in primary human NK and T cells (34). In human colon carcinoma cell lines, IL-13 induced phosphorylation and activation of JAK2 (35), whereas phosphorylation of tyrosine kinase and JAK2 was induced in human monocytes (29). However, both IL-13 and IL-4 induce the phosphorylation and nuclear translocation of the transcription factor STAT6 (16). The eventual involvement of these signaling molecules in the observed up-regulation of CysLT1R by IL-13 or IL-4 remains to be elucidated. The promotor region of the CysLT1R gene is undefined at present, but our findings would predict that it contains STAT6-binding elements.

Monocytes and macrophages are believed to play a pivotal role at sites of inflammation, as they have the potential to activate other cell types of inflammatory lesion. Our findings provide evidence that the cytokines IL-13 and IL-4 can enhance the expression and function of CysLT1R, a receptor for a potent proinflammatory lipid mediator. In the context of a Th2 response, IL-13 and IL-4 may thus play a crucial role in the accumulation and activation of mononuclear cells within the inflammatory lesion.

In conclusion, our observations that Th2 cytokines can up-regulate CysLT1R expression suggest a mechanism by which IL-13 and IL-4 can modulate leukocyte function and particularly their responsiveness to LTD4, and thus possibly contribute to the pathogenesis of asthma and allergic diseases.

Acknowledgments
We thank Denis Gingras and Sylvie Turcotte for excellent technical assistance.

References