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IL-5 Up-Regulates Cysteinyl Leukotriene 1 Receptor Expression in HL-60 Cells Differentiated into Eosinophils

Maryse Thivierge,* Micah Doty,† Jeff Johnson,† Jana Staňková,* and Marek Rola-Pleszczynski‡

The cysteinyl leukotrienes, leukotriene (LT) C₄, LTD₄, and LTE₄, are lipid mediators that have been implicated in the pathogenesis of several inflammatory processes, including asthma. The human LTD₄ receptor, CysLT₁R, was recently cloned and characterized. We had previously shown that HL-60 cells differentiated toward the eosinophilic lineage (HL-60/eos) developed specific functional LTD₄ receptors. The present work was undertaken to study the potential modulation of CysLT₁R expression in HL-60/eos by IL-5, an important regulator of eosinophil function. Here, we report that IL-5 rapidly up-regulates CysLT₁R mRNA expression, with consequently enhanced CysLT₁R protein expression and function in HL-60/eos. CysLT₁R mRNA expression was augmented 2- to 15-fold following treatment with IL-5 (1–20 ng/ml). The effect was seen after 2 h, was maximal by 4 h, and maintained at 8 h. Although CysLT₁R mRNA was constitutively expressed in undifferentiated HL-60 cells, its expression was augmented 2- to 15-fold following treatment with IL-5 (1–20 ng/ml). The effect was seen after 2 h, was maximal by 4 h, and maintained at 8 h. Although CysLT₁R mRNA was constitutively expressed in undifferentiated HL-60 cells, its expression was not modulated by IL-5 in the absence of differentiation. Differentiated HL-60/eos cells pretreated with IL-5 (10 ng/ml) for 24 h showed enhanced CysLT₁R expression on the cell surface, as assessed by flow cytometry using a polyclonal anti-CysLT₁R Ab. They also showed enhanced responsiveness to LTD₄, but not to LTB₄ or platelet-activating factor, in terms of Ca²⁺ mobilization, and augmented the chemotactic response to LTD₄. Our findings suggest a possible mechanism by which IL-5 can modulate eosinophil functions and particularly their responsiveness to LTD₄, and thus contribute to the pathogenesis of asthma and allergic diseases. The Journal of Immunology, 2000, 165: 5221–5226.

L leukotrienes (LTs), which are derived through the 5-lipoxygenase pathway of arachidonic acid metabolism, are lipid mediators of inflammation and immediate hypersensitivity (1–3). The cysteinyl LTs (cysLTs), 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. They mimic many of the features of human asthma, including bronchoconstriction, mucus secretion, and bronchial hyperresponsiveness (4–7).

Blood eosinophils from asthmatic patients synthesize greater amounts of cysLTs than those from normal subjects (8–10), and eosinophils appear to be the predominant source of cysLTs in the persistent asthmatic lung (11). CysLTs, in turn, have in vitro chemotactic activity for human eosinophils (12). Moreover, although the antitumorconstrictor efficacy of antileukotriene drugs provided the main impetus behind their recent introduction in asthma therapy, clinical trials also provided surprising evidence for a hitherto unsuspected role of cysLTs in promoting persistent eosinophilia in the airways and blood of asthmatics (13, 14). LTD₄-induced eosinophil infiltration of the airway could be blocked by the early cysLT antagonist MK-571 (15). More recently, in human studies, blocking the LTD₄ receptor has been shown to reduce airway eosinophil numbers in mildly uncontrolled asthma (16), suggesting it may be a contributing factor in lung eosinophilia in asthma.

Eosinophils are known to be important effector cells in IgE-mediated allergic tissue reactions, including asthma, as well as in immune and inflammatory reactions against parasitic infections. This capacity is mediated by the secretion of a variety of immunoregulatory and inflammatory cytokines, such as GM-CSF, IL-1α, IL-3, IL-5, IL-6, IL-8, and TNF-α, and the chemokine macrophage-inflammatory protein-1α (reviewed in Ref. 17). Several cytokines, including IL-3, IL-5, and GM-CSF are reported to regulate the development of eosinophils from hematopoietic stem cells and to support their survival in vitro (18–21). IL-5 is one of the most important regulators of many eosinophil functions including chemotaxis (22, 23), degranulation (24, 25), adhesion (26), and cytotoxicity for parasites (27). Moreover, treatment of normal eosinophils with IL-5, IL-3, or GM-CSF enhanced their cysLT production in vitro (28–30). IL-5 is also synthesized by Th2 lymphocytes and mast cells in the asthmatic airway (31–33) and is detectable in the plasma of symptomatic asthmatics (34). The importance of IL-5 in pathogenesis is underscored by the findings that an anti-IL-5 Ab completely blocks eosinophilic airway infiltration in a mouse model of asthma (35). Furthermore, mice with targeted disruption of the IL-5 gene are unable to develop eosinophilic inflammation of the airways or airway hyperreactivity after allergen challenge (36). Thus, IL-5 can regulate eosinophilic inflammation of the airways by modulating multiple functions of eosinophils.

Recently, Lynch et al. (37) reported the successful cloning and expression of a high-affinity cell surface human LTD₄ receptor (CysLT₁R) which belongs to the G protein-coupled receptor family.
This was soon followed by the publication of Sarau et al. (38), who also reported the cloning and characterization of a CysLTR.

The characterization of leukotriene receptors in human eosinophils, however, is hampered by the limited numbers of eosinophils available from peripheral blood. The promyelocytic leukemia HL-60 cell line can be differentiated toward the eosinophil phenotype (HL-60/eos) when cultured in alkaline medium with sodium butyrate (39, 40). We recently showed that HL-60/eos cells express specific functional LTD4 receptors (41). When they are differentiated toward the eosinophil phenotype, HL-60 cells present several markers and characteristics of mature eosinophils (39, 40). Furthermore, culture in the presence of butyric acid renders HL-60 cells responsive to IL-5, owing to induction of surface IL-5 receptor expression (42). Thus, HL-60/eos cells provide a useful model with which to study the modulation of CysLT,R. In the present study, we set out to determine whether the cytokine IL-5 could modulate the expression of CysLT,R on HL-60/eos cells.

Materials and Methods

Cell culture and stimulation conditions

HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS (HyClone, Logan, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere with 5% carbon dioxide at 37°C. Differentiation toward the eosinophil phenotype was induced as previously reported (40) by suspending 5 × 10⁶ cells/ml in RPMI 1640 medium containing 10% FBS, antibiotics (as above), 25 mM HEPPS, and 0.5 mM l-butylric acid at pH 7.8. The medium was changed every 3 days. Eosinophil-differentiated HL-60 cells are referred to as HL-60/eos in this text.

RT-PCR

Total RNA was extracted from cells by the guanidine-thiocyanate method described (43). A 10-μg pellet of RNA was converted to cDNA by the reverse transcriptase enzyme reaction (AMV transcriptase-reverse; Promega, Madison, WI) in a total volume of 20 μl. PCR was performed in a final volume of 100 μl containing 5 μl of RT reaction product. Samples were placed in a thermal cycler (DNA thermal cycler; Perkin-Elmer, Norwalk, CT) for 34 cycles of 2 min denaturation at 94°C, 30 s annealing at 94°C, 30 s annealing at 94°C, 90 s extension at 72°C, and 90 s extension at 72°C. Following a final 5-min extension at 72°C.

CysLT,R was amplified with the primers derived from the published cDNA sequence for CysLT,R (37), 5′-CGGGATCCGATGAAACAG GAGCGGTGAC′-3′ as sense and 5′-CCGGAATTCAGTATCATTCTAC′-3′ as antisense. Samples were subjected to parallel amplification of the constitutively expressed, housekeeping gene, GAPDH using the following primers: 5′-CTAGAGATGCTGTCTGCTG-3′ as sense and 5′-AAGGCG GAGCGGTGAC′-3′ as antisense. A 10-μl aliquot from each PCR was allowed to migrate by electrophoresis in a 1% agarose gel. The CysLT,R-amplified fragment contained 1014 bp. The gel was then colored with ethidium bromide and photographed under UV transillumination. No PCR products were obtained when reverse transcriptase was omitted, indicating that there was no DNA contamination.

Northern blot analysis

In selected experiments, Northern blot analysis was performed to assess more quantitatively the steady-state levels of CysLT,R mRNA. Total cellular RNA (20 μg/sample) was separated by electrophoresis on 1% agarose and transferred onto a Hybond-N (Amerham, Oakville, Ontario, Canada) membrane for Northern blot analysis. The cDNA corresponding to the whole coding sequence of human CysLT,R was amplified by PCR from DNA of human monocytes. Control hybridizations were performed with the human GAPDH cDNA. Membranes were prehybridized and hybridized overnight at 42°C in a mixture containing 20 mM Tris (pH 7.4), 600 mM NaCl, 8 mM EDTA (pH 8.0), 0.1% sodium pyrophosphate, 0.2% SDS, and 100 μg/ml heparin; hybridization was performed overnight at 42°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 7.0); once with 0.1× SSC/0.1% SDS at 60°C for 60 min, and then rinsed at room temperature with 0.1× SSC. The membranes were exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) with intensifying screens at −80°C.

Flow cytometry

The expression of CysLT,R in HL-60 and HL-60/eos cells was assessed using a polyclonal anti-CysLT,R Ab directed against the carboxyl-terminal portion of the receptor. The Ab was raised against a peptide corresponding to amino acids 318–337 of the C terminus of human CysLT,R. The peptide was conjugated to keyhole limpet hemocyanin, and rabbits were immunized with 0.2 mg of Ag in CFA. Boosts were done with 0.1 mg of Ag in IFA at 3-week intervals. Anti-CysLT,R Ab from serum obtained after the fifth boost was purified using a peptide-Sepharose affinity column. The Ab labeled CysLT,R-transfected cells, but not cells transfected with the LTβR receptor or vector alone. Preincubation of the Ab with the cognate peptide prevented all labeling.

For flow cytometry studies, HL-60 cells were washed with PBS and fixed with 2% paraformaldehyde for 15 min at room temperature followed by permeabilization with 0.1% Triton X-100 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-CysLT,R Ab (or with control, nonpertinent Ab. Cells were then washed with cold PBS and incubated for 30 min at 4°C with FITC-conjugated goat anti-rabbit IgG (Bio/CAN Scientific, Mississauga, Ontario, Canada). Finally, cells were washed again and resuspended in PBS before single-color immunofluorescence analysis of 5000 cells was performed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). A 1/2000 dilution of the anti-CysLT,R antisera was used in all cytometry studies.

Intracellular calcium mobilization

For Ca²⁺ mobilization assays, 3 × 10⁵ cells were loaded in HBSS (Life Technologies, Rockville, MD), containing 350 μg/ml NaHCO₃ and 10 mM HEPES (pH 7.0) with the calcium indicator fura-2-acetoxyethyl ester (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 in a SLM/Aminco spectrofluorometer (SLM Instruments, Urbana, IL). The concentration of extracellular Ca²⁺ was brought to 1.5 mM by addition of a solution of CaCl₂ into the cuvette 10 min before recordings. Maximal cell fluorescence (Fₐ₃₅₀max) was obtained by adding Triton X-100 to a final concentration of 0.3%. Minimum fluorescence (Fₐ₃₅₀min) was determined by subtracting the dilution of the chelator EGTA in Tris-HCl buffer (100 mM, pH 9.0) at 125 mM. Stimuli consisted of LTD₄, LTβ₂, and platelet-activating factor (PAF; Cayman Chemicals, Ann Arbor, MI).

Chemotaxis assay

HL-60/eos chemotactic activity was performed using Boyden chambers using a modified Boyden chamber chemotaxis assay. A volume of 200 μl of LTβ₃ or control medium was added to the lower chamber and 200 μl of HL-60/eos (6 × 10⁶) in RPMI 1640 supplemented with 2.5 μg/ml BSA was added to the upper chamber. 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 filter was disassembled and the upper side of the filter was scraped free of cells. Cells on the lower side were removed with 5 mM EDTA and centrifuged before counting on the FACScan.

Results

As a first step in assessing the effect of IL-5 on CysLT,R expression, we analyzed CysLT,R mRNA expression by semiquantitative RT-PCR in differentiated and undifferentiated HL-60 cells incubated for 8 h with graded concentrations of the cytokine. As illustrated in Fig. 1, IL-5 induced a concentration-dependent augmentation of CysLT,R mRNA expression in HL-60/eos cells. Whereas undifferentiated cells expressed constitutive CysLT,R mRNA, there was no modulation with IL-5. Although the amount of CysLT,R mRNA is generally low in HL-60/eos cells, Northern blot analysis was nevertheless performed to allow a more quantitative assessment of CysLT,R mRNA steady-state expression. As shown in Fig. 2A, IL-5 induced a time-dependent augmentation of CysLT,R mRNA expression as early as 2 h of treatment, which persisted at 8 h. Pretreatment of HL-60/eos cells with actinomycin

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D for 15 min, to block new RNA synthesis, blocked the IL-5-induced accumulation of CysLT₁ R mRNA (Fig. 2 B), suggesting a transcriptional mechanism in the regulation of CysLT₁ R expression by IL-5.

The next series of experiments examined whether transcription of CysLT₁ R mRNA was associated with an augmented expression of CysLT₁ R protein in HL-60/eos cells. Flow cytometry studies were performed using an affinity-purified polyclonal Ab directed against the C-terminal portion of CysLT₁ R. For these experiments, undifferentiated HL-60 and HL-60/eos cells were cultured for 24 h in the absence or presence of 10 ng/ml IL-5 before labeling with the Ab as described in Materials and Methods. As illustrated in Fig. 3, HL-60/eos readily expressed CysLT₁ R protein and the specificity of the antiserum was demonstrated by blocking of HL-60/eos labeling in the presence of the cognate peptide. No blocking was seen with an irrelevant peptide (data not illustrated). IL-5 treatment of HL-60/eos cells induced a markedly augmented expression of CysLT₁ R protein, as compared with untreated cells (Fig. 4). Although undifferentiated HL-60 cells transcribed CysLT₁ R mRNA at levels similar to HL-60/eos cells, they expressed lower levels of CysLT₁ R protein at their cell surface and could not modulate it in response to IL-5.

The augmented expression of CysLT₁ R protein by IL-5 treatment of HL-60/eos cells was dependent on both duration of treatment and concentration of IL-5. As illustrated in Fig. 5 A, enhanced CysLT₁ R protein expression was detectable by 12 h of treatment.
peaked at 24 h, and was still evident at 48 h. Ten nanograms of IL-5 per milliliter induced the strongest increase in CysLT₁R expression (Fig. 5B).

We finally investigated whether the IL-5-induced up-regulation of CysLT₁R expression was associated with an augmentation of the biologic responsiveness to LTD₄. Receptor function was first evaluated by mobilization of intracellular calcium upon stimulation with LTD₄. As illustrated in Fig. 6A, undifferentiated HL-60 cells showed a small response to LTD₄, consistent with their basal expression of CysLT₁R, but not to PAF. HL-60 eosinophils, in contrast, readily responded to both LTD₄ and PAF. The response to LTD₄, but not to PAF, was totally blocked by the selective CysLT₁R antagonist MK-571 (Biomol, Plymouth Meeting, PA). Moreover, the magnitude of the calcium flux was increased in HL-60 eosinophils cultured for 24 h in response to LTD₄, but not to PAF. As shown in Fig. 6B, the enhanced responsiveness of IL-5-treated HL-60 eosinophils to LTD₄ was associated with both an increased maximal response (Δ[Ca²⁺]ᵢ = 485 ± 109 nM for control cells vs 978 ± 168 nM for IL-5-treated cells, n = 3) and a reduced EC₅₀ value (4.1 ± 1.0 nM vs 1.3 ± 0.3 nM) for LTD₄. In counterpart, IL-5 had no significant effect on the

**FIGURE 5.** Time- and concentration-dependent modulation of CysLT₁R expression by IL-5. HL-60/eos cells were incubated for indicated periods in the presence of 10 ng/ml IL-5 (A) or for 24 h in the presence of graded concentrations of IL-5 (B) before labeling with either nonpertinent Ab (dotted line) or anti-CysLT₁R Ab followed by FITC-conjugated goat anti-rabbit Ab (untreated cells, thin line; treated cells, thick line). Treatment with IL-5 did not alter staining with the nonpertinent Ab (data not shown). Illustrated is one representative set of experiments out of three.

**FIGURE 6.** Intracellular calcium mobilization. [Ca²⁺]ᵢ was determined using the fluorescent dye fura-2-AM. A, Undifferentiated HL-60 cells or eosinophil-differentiated HL-60 (HL-60/eos) cells, cultured for 24 h in the absence or presence of IL-5 (10 ng/ml), were loaded with fura-2-AM and stimulated with 40 nM LTD₄ or 10 nM PAF in the presence or absence of the selective CysLT₁R antagonist MK-571 (1 μM). B, Eosinophil-differentiated HL-60 (HL-60/eos) cells, cultured for 24 h in the absence or presence of IL-5 (10 ng/ml), were loaded with fura-2-AM and stimulated with 1, 10, or 100 nM LTD₄.

C, Combined data from three separate experiments illustrating Δ[Ca²⁺]ᵢ values following stimulation of cells with 40 nM LTD₄, 20 nM LTD₄, or 10 nM PAF.
response of HL-60/eos cells to other stimuli, namely, LTB₄ or PAF (Fig. 6C).

CysLT₁R function was also assessed by the chemotactic response of HL-60/eos to LTD₄ following treatment with IL-5. Fig. 7 shows the significantly augmented response of IL-5-treated cells to LTD₄. Both an increased number of migrating cells and a reduced EC₅₀ value (8.8 ± 2.4 nM for untreated cells vs 0.4 ± 0.1 nM for IL-5-treated cells) for LTD₄ were observed.

These observations suggest that the augmentation of CysLT₁R expression following stimulation with IL-5 is accompanied by an enhanced functional activity of the receptor.

Discussion

The cysteinyl leukotrienes are lipid mediators that have been implicated in the pathogenesis of several inflammatory processes, including asthma. We previously showed that HL-60 cells developed specific and functional LTD₄ receptors when induced to differentiate toward the eosinophil lineage (41). Since the human LTD₄ receptor CysLT₁R was recently cloned and characterized, we undertook the present work to study the potential modulation of CysLT₁R expression in HL-60/eos cells by IL-5, an important regulator of eosinophil function. Our results show, for the first time, that the cytokine IL-5 rapidly up-regulates CysLT₁R mRNA expression. Moreover, IL-5-induced augmentation of CysLT₁R mRNA levels is associated with enhanced CysLT₁R protein expression in HL-60/eos cells, as illustrated with the use of anti-CysLT₁R Ab. These newly induced receptors are functional since pretreatment of HL-60/eos cells with IL-5 is associated with an augmented responsiveness to the ligand LTD₄ in terms of intracellular calcium mobilization and chemotactic activity.

The findings that IL-5 can modulate CysLT₁R expression on eosinophilic cells has potential implications in inflammation and asthma. There is substantial evidence linking cytokines with tissue eosinophilia. In allergic inflammatory diseases, the release of several eosinophil-priming cytokines such as IL-5, GM-CSF, and IL-3 is increased (44, 45). An association between the expression of lymphocyte Th2 cytokines, particularly IL-5, and asthma has been demonstrated (31). Elevated levels of IL-5 have been detected in bronchoalveolar lavage fluid obtained 24–48 h following local Ag challenge.

Excessive production of eosinophils and their subsequent invasion of the airways and other target organs are characteristic features of asthma and allergic diseases. Although IL-5 seems to have little effect on the migration of eosinophils into tissues, it has been shown to prime human eosinophils to respond to other stimuli such as RANTES and IL-8 (46). Intravenous IL-5 dramatically enhances the local accumulation of eosinophils induced by intradermal eotaxin or LTB₄ in guinea pigs (47). Circulating, rather than local, pulmonary IL-5 is required for the development of Ag-induced airways eosinophilia, possibly through its action at the level of the bone marrow (48). Since undifferentiated HL-60 cells are arrested at the promyelocytic stage of myelopoiesis, it is interesting to observe that CysLT₁R expression is already found in myeloid precursor cells, although additional studies with primary bone marrow-derived cells will be necessary to confirm this possibility. These observations are in contrast to those concerning the receptors for PAF (49) or LTB₄ (our unpublished observation) which require HL-60 cell differentiation to be expressed and functional. In fact, eosinophilic differentiation does not even induce responsiveness to LTB₄ (Fig. 6), whereas it induces responsiveness to PAF and augments responsiveness to LTD₄. Butyric acid is thought to activate genes by blocking the actions of deacetylases, thereby inhibiting histone binding to DNA due to increased histone acetylation (50). This may lead to the observed induction of IL-5Rα expression during eosinophil differentiation with butyric acid (42). Signaling through IL-5Rα/IL-5Rβ leads to activation of Janus kinase 2 and subsequent phosphorylation and dimerization of STAT factors. IL-5 also activates Lyn, Syk, Raf-1, and PI3 kinases and SHP-2 tyrosine phosphatase, which are differentially involved in eosinophil growth and differentiation, survival, up-regulation of adhesion molecules or priming for degranulation, and production of superoxide and LTC₄ (51). The eventual involvement of these signaling molecules in the observed up-regulation of CysLT₁R by IL-5 remains to be elucidated.

Anti-inflammatory glucocorticoid therapy reduces IL-5 expression and suppresses airway eosinophilia in asthma (34, 52). Thus, priming of eosinophils with cytokines such as IL-5 and the increase in CysLT₁R expression in the airways of asthmatics may contribute to eosinophil influx and activation.

In summary, the eosinophil-differentiated HL-60 cells can be used as a model for the study of CysLT₁R regulation. Our findings suggest a possible mechanism by which IL-5 can modulate eosinophil function and particularly their responsiveness to LTD₄, and thus possibly contribute to the pathogenesis of asthma and allergic diseases.

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References
