

Expression of functional cysteinyl leukotriene receptors by human basophils

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Background: Synthesis of leukotriene (LT) C₄ by basophils and mast cells is an important component of IgE-mediated inflammation, resulting in increased levels of the cysteinyl leukotrienes (cysLTs) LTC₄, LTD₄, and LTE₄. Receptors for cysLTs are expressed on a variety of peripheral blood leukocytes, but of interest, they are also expressed on cells that synthesize LTC₄, such as eosinophils and mast cells. **Objective:** We examined human basophils for expression and function of cysLT receptor type 1 (cysLT1) and cysLT receptor type 2 (cysLT2).

Methods: Basophils were purified from human blood and analyzed by means of RT-PCR, Western blotting, and flow cytometry for receptor expression. Basophils were also examined for functional responses to LTD₄, including cytosolic calcium flux, histamine release, viability, and chemotaxis.

Results: We found that basophils express mRNA for cysLT1 and cysLT2. CysLT1 and cysLT2 were also detectable by means of flow cytometry, but only cysLT2 was detectable by means of Western blotting with available antibodies. Increases in cytosolic calcium induced by LTD₄-stimulated basophils were inhibited by the cysLT1 receptor antagonist zafirlukast, confirming the presence of functional cysLT1 receptors on basophils. There was no significant effect of LTD₄ on histamine release; however, LTD₄ decreased CD95 (Fas) expression on basophils in several-day cultures.

Conclusions: We have demonstrated that basophils express the cysLT receptors cysLT1 and cysLT2, and some functional responses to LTD₄ can be observed. (*J Allergy Clin Immunol* 2005;116:80-7.)

Key words: Basophil, cysteinyl leukotriene receptor type 1, cysteinyl leukotriene receptor type 2, mRNA, viability, chemotaxis

Basophils are central to IgE-mediated inflammation, are found at increased levels in tissues of allergic subjects,¹ and are further increased after allergen challenge.¹⁻³

Abbreviations used

CysLT: Cysteinyl leukotriene
CysLT1: Cysteinyl leukotriene receptor type 1
CysLT2: Cysteinyl leukotriene receptor type 2
FMLP: Formyl-methionyl-leucyl-phenylalanine
LT: Leukotriene
PAGCM: Piperazine-N,N'-bis-2-ethanesulfonic acid buffer containing 0.003% normal human serum, 0.1% glucose, 1 mM MgCl and 1 mM CaCl
PE: Phycoerythrin
PIPES: Piperazine-N,N'-bis-2-ethanesulfonic acid
TBST: 50 mmol/L Tris buffer, 0.15 mol/L NaCl, 0.1% Tween-20

Cross-linking high-affinity IgE receptors with allergens leads to generation of leukotriene (LT) C₄,⁴ which is converted to LTD₄ and then to LTE₄.^{5,6}

The cysteinyl leukotrienes (cysLTs) LTC₄, LTD₄, and LTE₄ are increased in the airways of asthmatic subjects,⁷ with the ability to induce bronchoconstriction, vascular permeability, and mucus secretion.⁸⁻¹² CysLTs are well established as mediators of allergic rhinitis and asthma on the basis of the efficacy of antileukotriene therapy,¹³⁻¹⁵ and they might play a role in the accumulation of inflammatory cells to allergic tissue. Eosinophils and CD34⁺ cells, which express cysLT receptors, are found at increased numbers in allergic tissue and have been shown to migrate in response to LTD₄.¹⁶⁻¹⁸ Furthermore, cysLTs have been shown to play a role in survival of eosinophils¹⁹ and proliferation of eosinophil-basophil progenitor cells.²⁰ Basophil numbers have been shown to increase in the airway after inhalation of LTE₄,⁹ supporting the hypothesis that cysLTs might have a direct effect on basophil accumulation in the airways.

CysLT receptors are expressed on a variety of peripheral blood leukocytes,²¹ but of interest, they are also expressed on cells that synthesize LTC₄, such as eosinophils²² and mast cells.²³ The expression of cysLT receptors has not been evaluated on basophils, and efforts to do this have been hampered by the limited number of basophils that can be purified from peripheral blood. Because cysLT1 receptors are expressed on the shared eosinophil and basophil progenitor cells, CD34⁺ cells,¹⁷ and both cysLT1 and cysLT2 receptors are present on mature eosinophils,²² we speculated that basophils might also express cysLT receptors. Basophils were therefore evaluated for cysLT1 and cysLT2 message, protein, and functional responses to LTD₄.

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METHODS

Basophil purification

Basophils were obtained from residual cells of donors undergoing leukapheresis, and cells were enriched with countercurrent-flow elutriation, followed by Percoll density gradients, as previously described.²⁴ Briefly, cells were cultured overnight in RPMI 1640 containing 25 mM HEPES and L-glutamine and 30 pg/mL recombinant human IL-3 and were further purified the following day by means of negative selection with an antibody cocktail (Stem Cell Technologies, Vancouver, British Columbia, Canada) and magnetic columns (Miltenyi Biotec, Auburn, Calif). Basophils were also obtained from the peripheral blood of normal donors and atopic asthmatic donors by means of direct venipuncture. Cells were then enriched with a double Percoll step gradient (1.066/1.079 g/mL) and purified with Stem Cell reagents and Miltenyi columns. Normal donors had negative skin test results to a panel of 19 allergens and were not using regular medication. Atopic asthmatic donors demonstrated a skin wheal of greater than 2 mm to at least 1 of the 19 allergens, had methacholine PC₂₀ values of less than 16 g/mL,²⁵ and were using no regular medications except intermittent β_2 -agonist for the treatment of asthma.

Eosinophil purification

Eosinophils and neutrophils obtained from residual cells of normal donors undergoing leukapheresis were isolated by means of centrifugation on Percoll discontinuous density gradients, followed by lysis of red blood cells. Eosinophils were purified by means of negative selection with magnetic beads conjugated with anti-human CD16 antibody (Miltenyi Biotec Inc).²⁶

RT-PCR

Oligonucleotide primers for human cysLT1 (sense: 5'-CTGCCACATGCCATGACACTA and antisense: 5'-ATAGACCACACGAGAGGCAGT) and cysLT2 (sense: 5'-CGTTTCCTGGCAATGGTTCA and antisense: 5'-GCCCAACCAAGGCAATATA) were synthesized, resulting in PCR products of 216 bp and 222 bp for cysLT1 and cysLT2, respectively. Total RNA was isolated, treated with DNase, and reverse transcribed to complementary DNA with murine leukemia virus reverse transcriptase and poly-T tail primer (oligo d[T]15). Complimentary DNA PCR was performed with Taq polymerase with the thermocycle profile consisting of an initial 1-minute denaturation at 94°C and then 35 cycles of 30 seconds of denaturation at 94°C, 1 minute of annealing at 50°C, and 1 minute of polymerization at 72°C and, finally, a 10-minute extension at 72°C. Reverse transcription of eosinophil RNA served as a positive control, water served as a negative control, and internal reverse transcriptase controls used basophil cDNA with oligonucleotide primers for human β -actin (sense: 5'-TGACGGGGTCACCCACACTGTGCCCATCTA and antisense: 5'-CTAGAAGCATTGCGGTGG). Some reactions were performed without addition of reverse transcriptase as a control for contaminating DNA. RNA was isolated from basophils of greater than 98% purity, with the other 2% being contaminants not binding to the column matrix during the negative selection process. RNA was isolated from an equal number of column contaminants (containing monocytes, lymphocytes, neutrophils, and 1% to 5% basophils) and underwent RT-PCR in parallel with basophil RNA to address the possibility that these contaminating cells were the sole contributors of mRNA for cysLT1 and cysLT2.

Western blotting

For determination of cysLT1 and cysLT2 receptors, 1×10^6 basophils were lysed in ESB sample buffer and boiled for 5 minutes. Cell lysates were electrophoresed on 10% Tris-glycine-polyacryla-

mid gels for 90 minutes at 145V, and then gels were transferred to supported nitrocellulose membranes with a Trans Blot (Novex Invitrogen, Carlsbad, Calif). The membranes were immersed overnight in 50 mmol/L Tris buffer, 0.15 mol/L NaCl, and 0.1% Tween-20 (TBST) containing 5% BSA. Immunoreactive proteins were detected by incubating with polyclonal rabbit anti-human cysLT1 and cysLT2 antibodies (Caymen Chemical, Ann Arbor, Mich) diluted 1:6000 and 1:2000, respectively, in TBST containing 5% BSA with and without specific blocking peptides for cysLT1 and cysLT2 (Caymen Chemical) at a concentration of 10 μ g/mL and 4 μ g/mL, respectively, for 90 minutes at room temperature. After washing, the membranes were incubated with horseradish peroxidase-conjugated goat-anti-rabbit antibody for 1 hour at room temperature. After washing again, membrane-bound anti-rabbit Ig antibody was visualized with ECL Western blotting detection reagents and Hyper-ECL luminescence detection film (Amersham, Piscataway, NJ).

Flow cytometry

Flow cytometry was carried out on purified basophils with double staining for Fc ϵ RI α and cysLT1 or cysLT2 and on eosinophils with single staining for cysLT1 or cysLT2. Basophils were washed with FACS buffer (0.1% sodium azide-PBA with 1% BSA) and blocking buffer (5% normal mouse serum and 5% normal human serum in FACS buffer). Cells were then incubated with phycoerythrin (PE)-conjugated mouse monoclonal anti-human Fc ϵ RI α or PE-conjugated mouse IgG isotype control antibodies (eBioscience, San Diego, Calif), washed with FACS buffer, and resuspended in fixation medium A and then permeabilization medium B (Caltag, Burlingame, Calif). Cells were then incubated with polyclonal rabbit anti-human cysLT1 or cysLT2 (1:1000 dilution) antibodies (Caymen Chemical), washed, and incubated with biotinylated mouse monoclonal anti-rabbit IgG or biotinylated mouse IgG isotype control (1:50 dilution) antibodies before incubating with streptavidin-PerCP. FACS lysing solution was added, and cells were stored in 1% paraformaldehyde-PBS. The detection and analysis of immunostaining was performed with FACS Scan (Becton Dickinson, San Jose, Calif) and compared between groups of donors by using the unpaired *t* test.

Cytosolic calcium measurements

Basophils in RPMI 1640 were labeled with fura 2-acetoxymethyl ester (Molecular Probes, Eugene, Ore) and examined for calcium changes by means of digital video microscopy with techniques previously described.²⁷ Ratio measurements on 30 to 100 cells per condition were analyzed. The optimal concentration for LTD₄ (Caymen Chemical) was determined in pilot experiments, and 30 nM was used in subsequent experiments. To examine the specificity of this response, we preincubated basophils for 5 minutes in 300 nM cysLT1 antagonist (zafirlukast) and then stimulated with 30 nM LTD₄. Formyl-methionyl-leucyl-phenylalanine (FMLP), 1 μ M, was used as a positive control for a cytosolic calcium response.

Histamine release

Histamine release was performed as described previously.^{28,29} Basophils were stimulated with LTD₄ alone and in combination with anti-IgE and the cysLT1 receptor antagonist SKF104353, its inactive analogue (SKF104373), and a partial cysLT2 antagonist (BAY-u9773). LTD₄ was added either 0 or 5 minutes before the addition of anti-IgE antibody. Each condition tested was performed in duplicate.

Chemotaxis

A migrational response was assessed in basophils by using a modification of the Boyden chamber assay with a 48-well microchemotaxis chamber (Neuro Probe, Inc, Cabin John, Md). Basophils

were adjusted to 4×10^6 /mL in piperazine-N,N'-bis-2-ethanesulfonic acid buffer containing 0.003% normal human serum, 0.1% glucose, 1 mM MgCl₂, and 1 mM CaCl₂ (PAGCM), and 50 μ L was placed in the upper well separated from 29 μ L of chemoattractant in the lower well by a 5- μ m pore size polycarbonate filter (Nuclepore Track-Etch Membrane; Whatman, Clifton, NJ). LTD₄ was dissolved in PAGCM. Assessments were carried out in triplicate, and diluent (PAGCM) and positive (stromal cell-derived factor 1) controls were included in each experiment. The multiwell chambers were incubated for 45 minutes at 37°C. The filters were then fixed and stained with Diff Quik (American Scientific Products, McGaw Park, Ill). The migrational response was determined by using 400 $\times$ magnification, and results were expressed as the number of cells counted in 10 random high-power fields. Flow cytometry was used to assay an LTD₄-induced change in forward-scatter characteristics of purified basophils. Basophils were warmed to 37°C and stimulated with 0.1 μ M LTD₄ for various lengths of time, removed from the water bath, and quickly assessed for their forward-scatter characteristic.

Viability assay

Basophils were incubated at 0.8 to 2.8×10^6 /mL in RPMI 1640 containing 10% FCS, 1% streptomycin-penicillin, and 0.03 pg/mL IL-3 with or without LTD₄ at a concentration of 0.01, 0.1, or 1.0 μ M for 3 days. In a separate set of experiments, purified basophils were incubated in RPMI 1640 containing 10% FCS, 1% streptomycin-penicillin, 0.03 pg/mL IL-3, and 0.01 μ M LTD₄ with or without a 10-minute preincubation with cysLT1 antagonist (0.05 μ M montelukast). Cells were harvested after 3 days of culture at 37°C and 5% carbon dioxide, and cytosin preparations were prepared on glass slides and fixed in acetone. Immunocytochemistry was carried out with anti-human CD95 (anti-Fas) mAb (Trevigen, Gaithersburg, Md) at a concentration of 10 μ g/mL and visualized with the alkaline phosphatase-antialkaline phosphatase method (DAKO, Glostrup, Denmark). Negative isotype controls were included for each slide. The percentage of CD95⁺ cells was enumerated from a count of 400 basophils under light microscopy and analyzed with ANOVA. Cell culture supernatants were stored at -70°C to assay for levels of IL-4 and IL-13 by means of ELISA (Biotrak System; Amersham Biosciences, Piscataway, NJ), with a sensitivity of 7 pg/mL.

A more detailed "Methods" section can be found at the Journal's Online Repository at www.mosby.com/jaci.

RESULTS

Gene expression determined by means of RT-PCR

Expression of cysLT1 and cysLT2 mRNA in basophils was analyzed by means of RT-PCR. Eosinophils have been shown to express mRNA for cysLT1 and cysLT2²² and therefore were used as a positive control. As determined by means of RT-PCR, basophils of at least 99% purity contained mRNA for cysLT1 and cysLT2 (Fig 1, A). There was no difference in RT-PCR band intensity in RNA from basophils at 98% purity, from basophils at 96% purity (accomplished by adding column contaminants back into the purified basophil sample), or in RNA isolated from the same number of column contaminants (which have been previously shown to be primarily lymphocytes and monocytes). This confirmed that the RNA from 1% contaminating cells could not account for all of the mRNA measured in the basophil samples of 99% purity, demon-

strating that basophils indeed contain message for cysLT1 and cysLT2.

Characterization of cysLT2 by means of Western blotting

A band at 58-kd was observed when basophil lysates were probed for either cysLT1 or cysLT2 (Fig 1, B). Western blot analysis of peripheral blood eosinophils revealed bands at 52 and 58 kd. In the presence of blocking peptide for cysLT2, there was a reduction in the intensity of the 58-kd band but not the 52-kd band. CysLT1 blocking peptide had no effect on the 52-kd or 58-kd bands measured in eosinophils or basophils.

Protein levels determined by means of flow cytometry

Peripheral blood eosinophils were used as a positive control for cell staining of cysLT1 and cysLT2. Basophils demonstrated variable expression of cysLT receptors, ranging from 1.0% to 81.6% and 0.8% to 80.6% for cysLT1 and cysLT2, respectively (Fig 2). The expression of cysLT1 and cysLT2 on basophils was significantly higher in donors with atopic asthma compared with that in atopic or normal subjects ($P < .05$).

Cytosolic calcium release

Basophils in 5 of 10 preparations responded to LTD₄ at a concentration of 0.03 μ M. In the 5 preparations in which a response could be observed, the increase in cytosolic calcium induced by LTD₄ was extremely transient (peak at 8 ± 2 seconds and 50% decay from peak at 9 ± 2 seconds) and occurred in less than $35\% \pm 5\%$ of basophils under observation (0.01 μ M LTD₄ generated approximately half the average response, and 0.1 μ M LTD₄ generated a response similar in magnitude to 0.03 μ M). Fig 3, A, shows an example of this transient response, as well as a comparison of the average response to LTD₄ versus the average response in the same preparation to 1 μ M FMLP (Fig 3, B). Under conditions in which the response to LTD₄ was either negative or occurred in a very small percentage of the observed basophils, stimulation with FMLP resulted in a strong cytosolic calcium response in 100% of the observed cells. In those preparations in which there was a response to LTD₄, preincubation of basophils with 300 nM zafirlukast completely inhibited the LTD₄-induced calcium response (data not shown).

Histamine release

LTD₄ alone did not induce histamine release or alter anti-IgE-induced histamine release at any concentration. This was true whether LTD₄ was added 5 minutes before anti-IgE or added with the anti-IgE antibody (Fig 4, A). Basophils were also stimulated with anti-IgE antibody in the presence of a cysLT1 antagonist (SKF104353) or its inactive analog (SKF104373; Fig 4, B) or a partial cysLT2 antagonist (BAY-u9773, data not shown). In the presence of either drug, histamine release was not altered, nor was there an effect on LTC₄ release. In these experiments LTC₄ releases averaged 57 ± 7 ng/10⁶ basophils, which

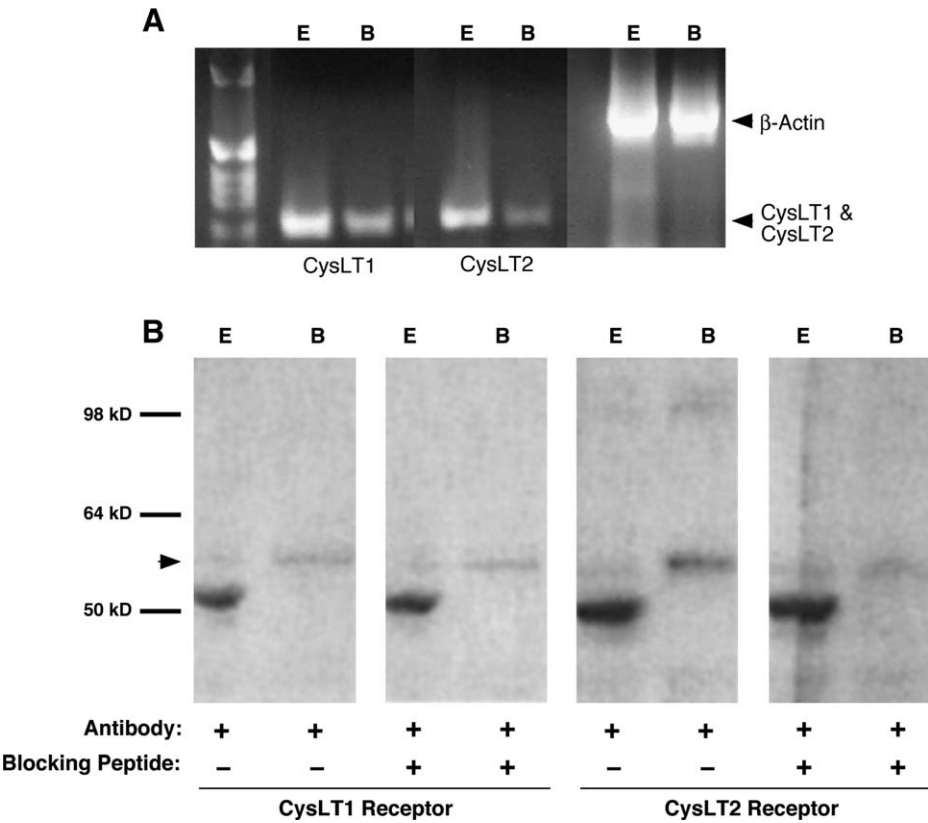


FIG 1. A, Ethidium bromide staining of RT-PCR product for cysLT1 receptor and cysLT2 receptor. B, Western blotting of cysLT1 receptor and cysLT2 receptor.

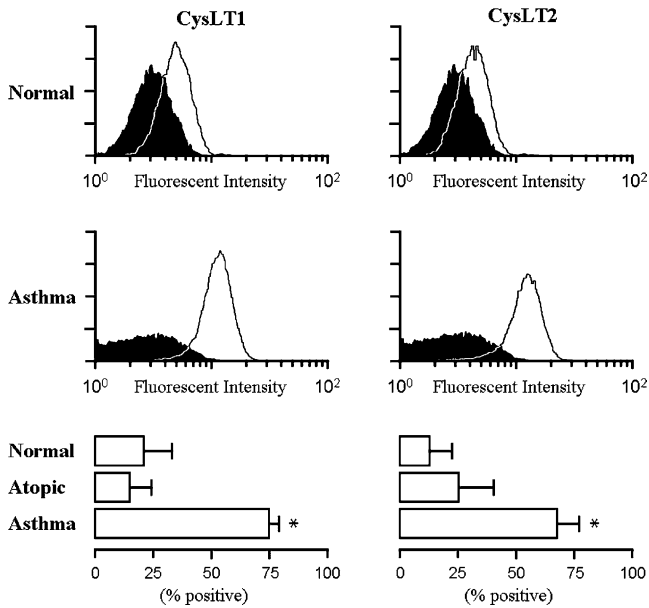


FIG 2. Flow cytometric analysis of cysLT1 receptor and cysLT2 receptor compared with isotype control (filled section). Bar graphs show the percentage of basophils (average $\pm$ SEM) staining positive for cysLT1 receptor and cysLT2 receptor.

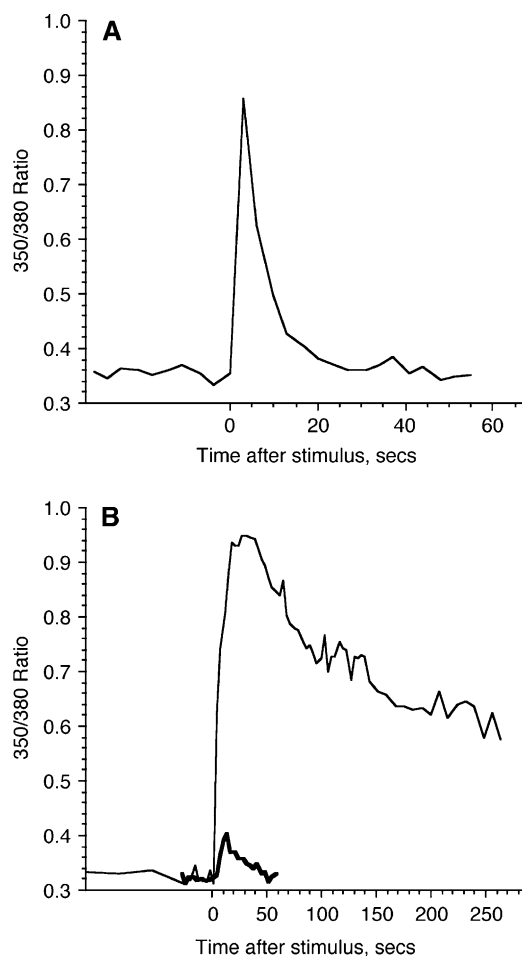


FIG 3. A, Cytosolic calcium release in an individual basophil after stimulation with LTD₄. **B**, Average response after stimulation with LTD₄ or FMLP.

would have resulted in a local average concentration of approximately 2 pM.

Chemotaxis and shape change

In 3 experiments, in a filter-based assay basophils did not migrate in response to LTD₄ but also poorly migrated in response to stromal cell-derived factor 1a, a known chemoattractant. This assay is often problematic for basophils, and therefore we examined shape change by means of flow cytometry, an assay sometimes used as a surrogate for chemokinesis. Fig 4, C, shows the kinetics of the forward-scatter change by using 0.1 μ M LTD₄. As found for the calcium response, the change in forward scatter was very transient, statistically returning to baseline after 1.5 to 2 minutes. For comparison, FMLP at 5 nM induced a peak net change in forward scatter of 30 units.

Viability

Culturing of basophils for 3 days in IL-3 at a suboptimal concentration of 0.03 pg/mL resulted in $24.6\% \pm 6.0\%$ of the basophils expressing Fas. Addition of LTD₄ at a concentration of 0.01, 0.1, and 1.0 μ M significantly decreased the percentage of basophils expressing Fas

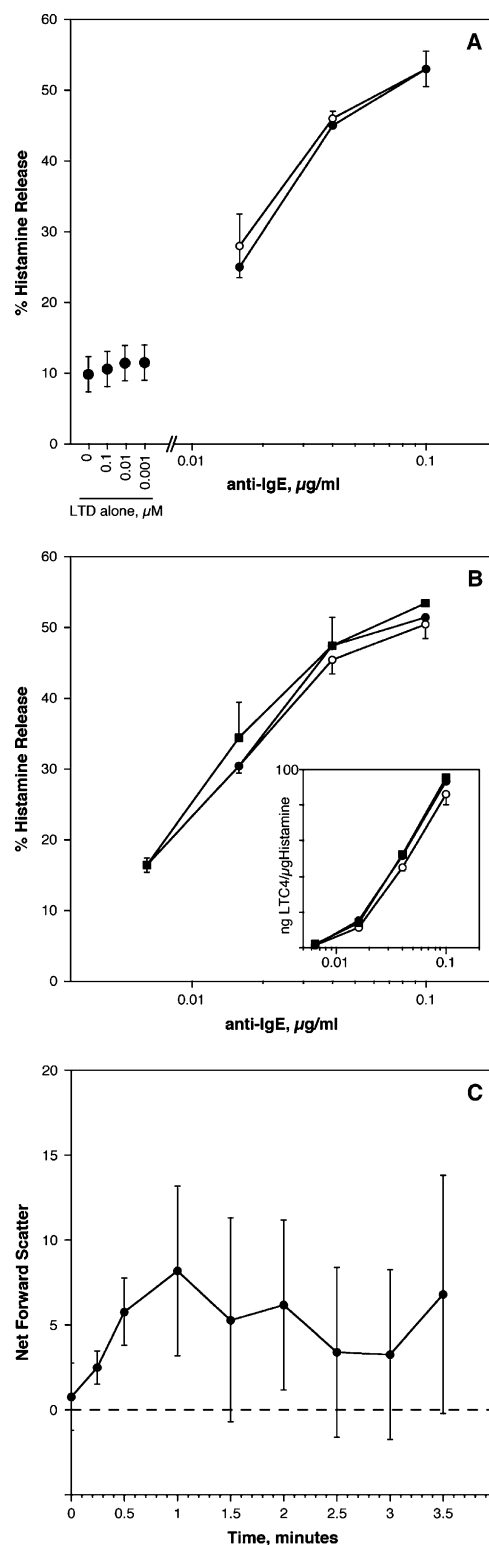


FIG 4. A, left, Percentage histamine release from impure basophils stimulated with LTD₄. **A**, right, Basophils stimulated with anti-IgE antibody (filled circles) with or without 0.1 μ M LTD₄ (open circles). **B**, Basophils stimulated with anti-IgE antibody (open circles) with or without 1 μ M SKF 104353 (filled circles) or SKF 104373 (filled squares). **C**, Forward scatter of basophils stimulated with 0.1 μ M LTD₄.

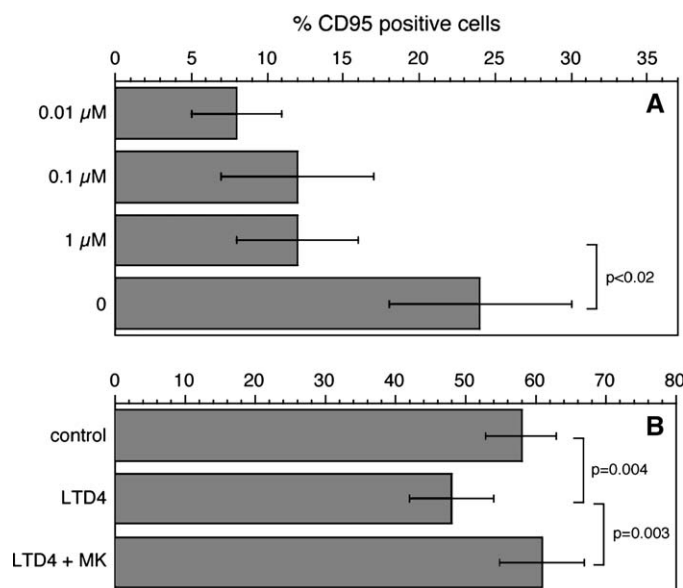


FIG 5. Percentage of basophils immunopositive for Fas/CD95 antibody after incubation with 0.03 pg/mL IL-3 with or without LTD₄ (A) and 0.03 pg/mL IL-3 with or without LTD₄, montelukast, or both (B).

($P < .02$; Fig 5, A). In a separate series of experiments, montelukast reversed the protection of LTD₄ for basophil expression of CD95 (Fig 5, B).

IL-4 and IL-13 generation

IL-13 levels were less than the level of detection in all baseline samples; however, they were measurable in 5 of the 11 samples incubated with LTD₄ (15.2 ± 8.6 pg/mL) and in 1 of the 11 samples incubated with LTD₄ plus montelukast (1.0 ± 1.0 pg/mL). IL-4 was less than the level of detection for all samples measured.

DISCUSSION

We have shown that basophils express mRNA for cysLT1 and cysLT2 and cell-surface receptor by means of flow cytometry. Our data obtained from Western blotting experiments are not as clear. Probing with the available polyclonal antibodies revealed bands at approximately 52 and 58 kd in eosinophil lysates, which was not the expected molecular weight. Different glycosylation or dimerization of receptors by mature leukocytes might explain our observation of higher-molecular-weight leukotriene receptors than previously reported. The calculated molecular mass based on the amino acid sequence of cysLT1 is reported to be 38.5 kd,^{30,31} and the monomeric recombinant protein is reported to be 42 kd,³⁰ although these authors also reported larger amounts of dimerized and oligomerized receptors in the presence of denaturing gel detergents. An earlier study with a different polyclonal antibody to cysLT1 has reported a single band at approximately 45 kd in CD34⁺ hematopoietic progenitors and a number of cell lines.¹⁷ Using the polyclonal antibodies in the current study, we would have expected to find a band

for cysLT1 near 50 kd and a band for cysLT2 between 53 and 55 kd (Cayman Chemical, personal communication). However, in both basophil and eosinophil lysates, we observed a 58-kd band when probing for cysLT1 and cysLT2. That we observed a reduction in the intensity of the band at 58 kd with cysLT2 blocking peptide suggests this band is indeed the cysLT2 receptor. There was no reduction in the intensity of the 58-kd band when a cysLT1 blocking peptide was applied, suggesting this band is not the cysLT1 receptor. These Western blotting results for cysLT2 are consistent with the flow cytometric data in basophils, together demonstrating that cysLT2 protein is detectable on the cell surface and in cell lysates. However, protein for cysLT1 is only detectable by means of flow cytometry with the available cysLT1 antibody.

Functional studies suggested the presence of a cysLT1 receptor. We observed an LTD₄-induced cytosolic calcium response, albeit one that was very weak by most standards. The magnitude of the average response was partially explained by the low percentage of cells showing a very transient response. Because the level of receptor expression would regulate calcium flux, the nature of the observed response could have been caused by low receptor levels in the blood donors (undergoing leukapheresis) whose atopic status was unknown. The response was ablated by zafirlukast, indicating that this was a response mediated by a cysLT1 receptor. Studies in other cells have found that cysLT1 and cysLT2 act through heterotrimeric guanosine triphosphate-binding proteins that mediate signaling through an increase of intracellular calcium.³²

A surprising second functional effect was found in culture experiments. Inclusion of LTD₄ reduced the frequency of Fas receptor (CD95) expression. Once again, this effect, although modest, was reversed by a cysLT1 receptor antagonist. The biologic significance of this effect

or its mechanism remained unclear. LTD₄ alone did not induce histamine release in basophils, but given the very weak cytosolic calcium response, this is perhaps not surprising. It remains possible that histamine release was not induced by exogenous LTD₄ because the concentrations tested were too low and that local concentrations *in situ* might exceed those tested.

We tested whether released LTC₄ could induce a feedback effect (positive or negative) by inclusion of a cysLT1 antagonist but did not observe a significant difference in histamine release. Because the selective cysLT1 antagonist did not have an effect, we then tested an antagonist acting on both cysLT1 and cysLT2 receptors, which also did not have an effect on histamine release. On this basis, we could tentatively conclude that the cysLT2 receptor did not mediate a feedback effect of released LTC₄. Given the considerable length of time required for basophil purification before cells were tested for functional responses, it is possible that the receptors were no longer present at optimal levels.

Basophils have been shown to accumulate in the airways of asthmatic subjects after inhalation of LTE₄,⁹ suggesting that cysLTs might play a role in the accumulation of basophils in allergic tissues. This could be a result of higher levels of cysLTs in the airways of asthmatic subjects, higher expression of cysLT receptors on basophils of asthmatic subjects (as we have demonstrated by means of flow cytometry), or both. Furthermore, zafirlukast has been shown to inhibit the infiltration of Alcian blue-positive cells (presumed to be basophils) in bronchoalveolar lavage fluid after segmental antigen challenge,¹³ confirming involvement of cysLT1. We hypothesized that this accumulation of basophils might be a result of increased viability, and our data showing decreased Fas expression after incubation with LTD₄ supports this possibility. Chemotaxis would be another potential mechanism resulting in accumulation of basophils in allergic tissues. Eosinophils and basophils share a common CD34⁺ hematopoietic progenitor. When CD34⁺ cells are stimulated with LTD₄, there is intracellular calcium mobilization and chemotaxis,¹⁷ and mature eosinophils have also been shown to be weakly chemotactic to LTD₄.³³ Although the chemotaxis assay was problematic for these basophil preparations, we did find a modest and transient change in forward-scatter characteristics by means of flow cytometry, and this behavior has been related to the ability of leukocytes to move in response to stimulation.

Our experiments have demonstrated that peripheral blood basophils express mRNA for cysLT1 and cysLT2, detectable levels of cysLT1 and cysLT2 protein, and functional responses to LTD₄. We have shown that despite the variable level of cysLT1 on basophils, there is indeed a functionally active receptor. Because 2 identified functional effects, increase of cytosolic Ca⁺⁺ and decreased expression of CD95, were fully reversed by the presence of a cysLT1 antagonist, we conclude that we have not identified a functional role for the cysLT2 receptor, despite its equivalent level of protein expression, as shown by

means of flow cytometry. Whether the observed level of response can account for the accumulation of basophils in allergic tissue remains to be seen.

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