

Human T_H2 cells respond to cysteinyl leukotrienes through selective expression of cysteinyl leukotriene receptor 1

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Background: Allergic asthma is characterized by reversible airway obstruction and bronchial hyperresponsiveness associated with T_H2 cell-mediated inflammation. Cysteinyl leukotrienes (CysLTs) are potent lipid mediators involved in bronchoconstriction, mucus secretion, and cell trafficking in asthmatic patients. Recent data have implicated CysLTs in the establishment and amplification of T_H2 responses in murine models, although the precise mechanisms are unresolved. **Objectives:** Preliminary microarray studies suggested that human T_H2 cells might selectively express cysteinyl leukotriene receptor 1 (*CYSLTR1*) mRNA. We sought to establish whether human T_H2 cells are indeed a CysLT target cell type. **Methods:** We examined the expression of *CYSLTR1* using real-time PCR in human T_H1 and T_H2 cells. We functionally assessed cysteinyl leukotriene receptor 1 protein (CysLT₁) expression using calcium flux, cyclic AMP, and chemotaxis assays. **Results:** We show that human T_H2 cells selectively express *CYSLTR1* mRNA at high levels compared with T_H1 cells after *in vitro* differentiation from naive precursors. Human T_H2 cells are selectively responsive to CysLTs in a calcium flux assay when compared with T_H1 cells with a rank order of potency similar to that described for CysLT₁ (leukotriene [LT] D₄ > LTC₄ > LTE₄). We also show that LTD₄-induced signaling in T_H2 cells is mediated through CysLT₁ coupled to G_{αq} and G_{αi} proteins, and both pathways can be completely inhibited by selective CysLT₁

antagonists. LTD₄ is also found to possess potent chemotactic activity for T_H2 cells at low nanomolar concentrations. **Conclusions:** These findings suggest a novel mechanism of action for CysLTs in the pathogenesis of asthma and provide a potential explanation for the anti-inflammatory effects of CysLT₁ antagonists. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Human, T_H1, T_H2, cysteinyl leukotrienes, chemotaxis, CYSLTR1, cysteinyl leukotriene receptor 1 protein, LTD₄

Allergic asthma is characterized by reversible airway obstruction and bronchial hyperresponsiveness to otherwise innocuous environmental antigens and is associated with inflammation of the airways involving numerous immune cell types and inflammatory mediators.^{1,2} In particular, the immune pathology observed is dominated by a T_H2-type response with increased expression of the classical T_H2 cytokines IL-4, IL-5, and IL-13, which might cause many of the hallmark features of allergic airways disease.³ Central to this T_H2 immune pathology is the allergen-specific T_H2 cell, which is the predominant source of T_H2 cytokines in asthmatic subjects, although the precise mechanisms by which the T_H2 phenotype develops in asthmatic subjects remain to be determined.⁴

Cysteinyl leukotrienes (CysLTs) are potent lipid mediators involved in the pathogenesis of a wide range of chronic inflammatory and immune disorders, including asthma, allergic rhinitis, atherosclerosis, and inflammatory bowel disease.^{5,6} The CysLT leukotriene (LT) C₄ is derived from arachidonic acid through cytosolic phospholipase A₂, 5-lipoxygenase, and LTC₄ synthase. LTC₄ is transported out of the cell and converted sequentially to LTD₄ and LTE₄ by the ubiquitous enzymes γ -glutamyl transpeptidase and aminopeptidase, respectively. The biological activities of the CysLTs are mediated through at least 4 G protein-coupled receptors, cysteinyl leukotriene receptor 1 protein (CysLT₁), CysLT receptor 2 protein, G protein-coupled receptor 17 (GPR17), and purinergic receptor P2Y₁₂ protein (P2Y₁₂), although the role of each of these receptors in CysLT action is incompletely understood.⁶⁻⁸ In asthmatic subjects the CysLTs are believed to be involved in bronchoconstriction, mucus secretion, and cell trafficking and have been shown to be chemotactic agents for eosinophils, hematopoietic progenitor cells, and monocytes.⁹⁻¹¹ In addition to their role in bronchoconstriction and innate cell recruitment to the lungs of asthmatic subjects, CysLTs play a role in the establishment and amplification of antigen-specific T_H2 cell-dependent immune responses in murine models of allergic airways disease.¹² Although the precise mechanistic role of CysLTs in the development of the adaptive T_H2 response is unclear, prior studies have focused on the role of CysLTs in pulmonary dendritic cell migration and maturation.¹³⁻¹⁵ Here we

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Abbreviations used

cAMP: Cyclic AMP
 CysLT: Cysteinyl leukotriene
 CysLT₁: Cysteinyl leukotriene receptor 1 protein
CYSLTR1: Cysteinyl leukotriene receptor 1 gene/mRNA
 GPR17: G protein-coupled receptor 17
 LT: Leukotriene
 P2RY12: Purinergic receptor P2Y₁₂ gene/mRNA
 P2Y₁₂: Purinergic receptor P2Y₁₂

report that human T_H2 cells selectively express functional CysLT₁ and that LTD₄ is a potent chemotactic agonist for T_H2 cells. T_H2 cells exhibit robust calcium signaling in response to all 3 CysLTs, with the most potent response observed with LTD₄ followed by LTC₄ and LTE₄, which is consistent with previous studies on CysLT₁.^{16,17} The calcium signaling in response to LTD₄ was completely blocked with the selective CysLT₁ antagonists MK571, montelukast, and zafirlukast. These findings suggest a novel mechanism of action for CysLTs in the pathogenesis of asthma and provide a potential explanation for the anti-inflammatory effects of CysLT₁ antagonists.

METHODS**Reagents**

Leukotrienes (LTC₄, LTD₄, and LTE₄) and CysLT₁ antagonists (MK571, montelukast, and zafirlukast) were purchased from Cayman Chemical (Ann Arbor, Mich). Recombinant chemokines (CXCL12 and CCL18) were purchased from R&D Systems (Abingdon, United Kingdom). Pertussis toxin, thapsigargin, and forskolin were purchased from Sigma-Aldrich (Dorset, United Kingdom). EDTA was purchased from Life Technologies (Paisley, United Kingdom).

Human T-cell isolation and differentiation

These studies were approved by the Research Ethics Committee of Guy's Hospital with informed consent. The donors used in this study were nonatopic, nonasthmatic male subjects (25–40 years) with no other chronic or acute illnesses at the time of venipuncture. Naive CD4⁺ T cells were isolated from peripheral blood by using magnetic positive selection of CD4⁺ cells (DynaL CD4 Positive Isolation kit; Invitrogen, Paisley, United Kingdom) followed by depletion of CD45RO⁺ memory cells, as described in detail previously.¹⁸ Naive T cells were stimulated with anti-CD3 (clone OKT3, ECACC, prepared in house) and anti-CD28 (clone 15E8; Sanquin Reagents, Amsterdam, The Netherlands) in the presence of cocktails of cytokines and antibodies to generate T_H1 and T_H2 cells, as described in detail previously.¹⁸ Every 7 days, T_H1/T_H2 differentiation was assessed by using intracellular cytokine staining, as described previously.¹⁸

RNA isolation, microarrays, and real-time reverse transcription PCR

Cells for RNA isolation were snap-frozen in liquid nitrogen and stored at –80°C. Total cellular RNA was isolated with the miRNeasy mini kit (Qiagen, Crawley, United Kingdom), according to the manufacturer's instructions. cRNA samples were prepared for microarray hybridization to GeneChip U133 plus 2 arrays, according to the manufacturer's instructions (Affymetrix, Santa Clara, Calif). Fragmented cRNA was hybridized to GeneChip arrays at 45°C for 18 hours. Arrays were washed and stained with streptavidin-phycoerythrin, according to the manufacturer's instructions, on the GeneChip fluidics station 450 (Affymetrix). Fluorescent signals were detected with the GeneChip scanner 3000. Images were analyzed with the GeneChip operating software (Affymetrix) to generate raw data as .cel files. Further analysis was

performed with the Partek Genomics Suite (Partek, St Louis, Mo) by using the gene expression workflow to identify differentially expressed genes. Briefly, robust multichip average preprocessing was performed, and genes differentially expressed between T_H1 and T_H2 cells were identified by using the Partek ANOVA model. One thousand seven hundred ninety-four genes were identified as differentially expressed with a *P* value of less than .05, including Benjamini-Hochberg step-up for false discovery rate. Data shown in Table I are the mean probe-set signals from 3 independent biological replicates for each condition. cDNA synthesis and real-time PCR were performed with TaqMan MGB probes (Applied Biosystems, Invitrogen, Paisley, United Kingdom), as described in detail previously.¹⁹ TaqMan probe sets used in this study were as follows: cysteinyl leukotriene receptor 1 (*CYSLTR1*), Hs00272624_s1; cysteinyl leukotriene receptor 2 (*CYSLTR2*), Hs00272624_s1; *IL5*, Hs00174200_m1; *IFNG*, Hs00174143_m1; and *18s* rRNA, 4319413E. Statistical analyses were performed by comparing gene expression levels relative to *18s* rRNA levels by using a 2-way repeated-measures ANOVA with Bonferroni post tests in GraphPad Prism version 5.0a software (GraphPad, Inc, La Jolla, Calif).

Calcium-signaling assay

Cultured human T_H1 and T_H2 cells were tested for calcium signaling when fully differentiated at days 21 and 28 in response to LTB₄, LTC₄, LTD₄, and LTE₄. Cells were resuspended at 200,000 cells/well in 100 μL of RPMI plus 20 mmol/L HEPES and plated onto a 96-well, black-wall, clear, flat-bottom assay plate (Costar), and an equal volume of loading buffer (Component A with 1X HBSS Buffer; FLIPR Calcium 4 Assay Kit; Molecular Devices, Eugene, Ore) was added. The plate was incubated for 45 minutes at 37°C and 5% CO₂. After incubation, the plate was centrifuged at 200 *g* for 5 minutes and transferred directly to a FlexStation 3 Microplate Reader (Molecular Devices) at 37°C. Varying concentrations of CysLTs were added as indicated. Where indicated, the selective CysLT₁ antagonist MK571 (100 nmol/L), montelukast (100 nmol/L), or zafirlukast (100 nmol/L) was added 5 minutes before adding ligand. In some experiments pertussis toxin (100 ng/mL) was added 16 hours before the assay was performed to inhibit G_αi. Where indicated, thapsigargin (1 μmol/L) or EDTA (2.5 mmol/L) was added 5 minutes before adding ligand. Results were analyzed with SoftMax Pro Software (Molecular Devices), and data are shown as a percentage of maximal response. Controls included a negative medium control of RPMI plus 20 mmol/L HEPES and a positive control of stromal cell-derived factor 1/CXCL12, and all experiments were performed with triplicate wells.

Cyclic AMP assay

Intracellular cyclic AMP (cAMP) levels were measured by luminescence with the HitHunter cAMP XS+ assay (DiscoverRX, Birmingham, United Kingdom). The assay was carried out according to the manufacturer's protocol for a 3-reagent addition. T_H2 cells were suspended in PBS at a concentration of 10 × 10⁶/mL and warmed to 37°C, and 1 nmol/L 3-isobutyl-1-methylxanthine (Sigma) was added to inhibit cAMP phosphodiesterases. The T_H2 cell suspension (2 × 10⁵ cells/well) was loaded into a 96-well plate, and 20 μmol/L forskolin was added to stimulate cAMP induction along with varying concentrations of LTD₄, as indicated. Where indicated, 100 nmol/L MK571 was added 5 minutes before adding ligand. Plates were incubated at 37°C for 15 minutes before cells were processed according to the kit's instructions, and cAMP signal was detected by means of luminescence measured 4 hours after cell lysis. Luminescence was detected by using a Flexstation 3 (Molecular Devices), and analysis of cAMP concentrations was performed with SOFTMax Pro software (Molecular Devices). Data are shown as a percentage of maximal response.

Chemotaxis assay

Chemotaxis assays were carried out by using a modification of a protocol²⁰ obtained from Andrew Luster (Massachusetts General Hospital, Harvard, Boston, Mass) by using 24-well 6.5-mm Transwell plates with a polycarbonate membrane insert filter and a pore size of 5.0 μm (3421; Corning, Corning, NY). *In vitro* cultured human T_H2 cells were resuspended at 1 × 10⁶/mL in RPMI buffer; rested for 4 hours in a low rIL-2 concentration (25 U/mL;

TABLE I. Expression of selected genes in preliminary microarray analysis

Gene name	Probe set ID	T _H 1, resting	T _H 1, activated	T _H 2, resting	T _H 2, activated
<i>CYSLTR1</i>	231747_at	349.4	179.2	1053	101.1
<i>CYSLTR2</i>	220813_at	6.549	6.517	10.02	7.413
<i>GPR17</i>	215225_s_at	3.993	4.112	3.956	4.067
<i>P2RY12</i>	224102_at	4.214	4.363	4.16	4.279
<i>PGK1</i>	200737_at	1902	1669	1956	1605
<i>TAF9B</i>	221618_s_at	206.6	74.86	312.6	137.3
<i>ZCCHC5</i>	1552935_at	6.926	9.382	6.134	6.99

Novartis, Horsham, United Kingdom); and then harvested, washed in PBS, and resuspended at 500,000 cells/100 μ L in chemotaxis buffer (RPMI + 20 mmol/L HEPES + 0.5% ultrapure BSA [Sigma, A7638]). Six hundred microliters of chemotaxis buffer with or without the chemokine or leukotriene of interest was loaded on the bottom of the Transwell. Each chemotactic agent (LTD₄, LTE₄, and the positive control stromal cell–derived factor 1) was tested in duplicate. By using tweezers, the Transwell filter was placed in the bottom well, and 100 μ L of cells was added and left to migrate for 2 hours in an incubator at 37°C 5% CO₂. After 2 hours, the plate was placed at 4°C for 15 minutes. Migrated cells were counted by using flow cytometry, with polystyrene beads as a reference (polystyrene 15- μ m beads; Polysciences, Warrington, Pa). A chemotactic index was calculated as the ratio of migration to chemoattractant compared with the basal migration to buffer alone.

RESULTS

Human T_H2 cells selectively express *CYSLTR1*

We have previously reported a method to generate highly polarized human T_H1 and T_H2 cells from naive precursors *in vitro* and have used this model extensively to investigate the lineage-specific functions of these cells.^{18,19,21} To identify novel lineage-specific molecules that might play a selective role in T_H2 cell function, we have previously performed a preliminary microarray-based study comparing T_H1 and T_H2 cells. Analysis of CysLT receptor mRNA expression in these samples revealed that *CYSLTR1* mRNA was highly and selectively expressed by T_H2 cells when compared with T_H1 cells ($P = .000692$, Table I). Expression of other potential CysLT receptors, *CYSLTR2*, *GPR17*, and *P2RY12*, was undetectable by means of microarray in both T_H1 and T_H2 populations (Table I). Intracellular cytokine staining of resting and activated T_H1 and T_H2 cells demonstrated that highly polarized T_H1/T_H2 populations were generated (Fig 1, A). On activation, at least 99% of the T_H1 cells express IFN- γ with minimal T_H2 cytokine expression. In contrast, the T_H2 cells express negligible IFN- γ , and a very high proportion express T_H2 cytokines (IL-4, 42%; IL-5, 46%; and IL-13, 85%). Quantitative real-time RT-PCR of highly differentiated T_H1 and T_H2 samples confirmed that the T_H1 and T_H2 cells express high levels of transcripts for the hallmark cytokines IFN- γ (T_H1) and IL-4, IL-5, and IL-13 (T_H2) on acute activation (Fig 1, B, and data not shown). Examination of *CYSLTR1* mRNA expression by using real-time RT-PCR confirmed that it is selectively expressed by resting T_H2 cells with 6.5-fold higher levels than seen in resting T_H1 cells (Fig 1, C). The selective expression of *CYSLTR1* was predominantly observed in cells before activation, with a substantial decrease in mRNA expression after acute activation of T_H2 cells (Fig 1, C). Real-time PCR analysis also confirmed that *CYSLTR2* mRNA was not expressed at high levels in either T_H1 or T_H2 cells (Fig 1, C). Examination of *CYSLTR1* mRNA expression during the time course of differentiation revealed that expression increased substantially during T_H2 differentiation (Fig 1, D),

suggesting that *CYSLTR1* expression is acquired during T_H2 differentiation rather than being expressed on naive T cells and selectively lost during T_H1 differentiation. This also indicated that expression of *CYSLTR1* by T_H2 cells is not merely caused by exposure to IL-4 because it requires at least 3 weeks of *in vitro* culture before high levels of expression are observed (Fig 1, D). The genes adjacent to *CYSLTR1* (ie, *PGK1*, *TAF9B*, and *ZCCHC5*) were not differentially expressed between T_H1 and T_H2 cells, suggesting that the T_H2-selective expression of *CYSLTR1* is not merely due to the gene being located in close proximity to other T_H2-specific genes (Table I).

T_H2 cell–specific calcium flux in response to CysLTs

CysLT signaling through CysLT₁ induces calcium flux in other cell types known to express the receptor. *In vitro* differentiated T_H1 and T_H2 cells were treated with LTD₄ and intracellular calcium responses were measured to determine whether the selective expression of *CYSLTR1* mRNA observed in T_H2 cells resulted in functional expression of CysLT₁ in these cells (Fig 2, A). LTD₄ induced a substantial calcium mobilization in T_H2 cells at concentrations similar to those reported for CysLT₁ in other systems (median effective concentration, approximately 1 nmol/L). A much weaker response to LTD₄ was observed in T_H1 cells at higher concentrations. This confirmed that human T_H2 cells selectively express a functional receptor for LTD₄. Treatment of T_H2 cells with LTC₄, LTD₄, and LTE₄ showed that all 3 CysLTs induced calcium mobilization, with LTD₄ being the most potent, followed by LTC₄ and LTE₄ (Fig 2, B). This rank order of potency (LTD₄ > LTC₄ > LTE₄) resembles the results of previous studies on CysLT₁ in other cell types, suggesting that T_H2 cells express functional CysLT₁.^{16,17} Interestingly, in contrast to the original studies with transfectants expressing CysLT₁, the potency of LTC₄ and LTE₄ is similar until higher concentrations of agonist are used (10–100 nmol/L), where LTC₄ induces more calcium flux. The CysLT₁ selective antagonists MK571 (100 nmol/L), montelukast (100 nmol/L), and zafirlukast (100 nmol/L) were used to treat T_H2 cells before treatment with 100 nmol/L LTD₄ (Fig 2, C) to confirm the identity of the CysLT-responsive receptor expressed by T_H2 cells. All 3 CysLT₁ antagonists almost completely inhibited calcium mobilization, confirming that the calcium flux in response to LTD₄ by human T_H2 cells is dependent on selective expression of functional CysLT₁.

CysLT₁ is coupled to both G α i and G α q G proteins in human T_H2 cells

T_H2 cells were preincubated with the G α i inhibitor pertussis toxin and treated with increasing concentrations of LTD₄ to further characterize the signaling mechanisms involved in

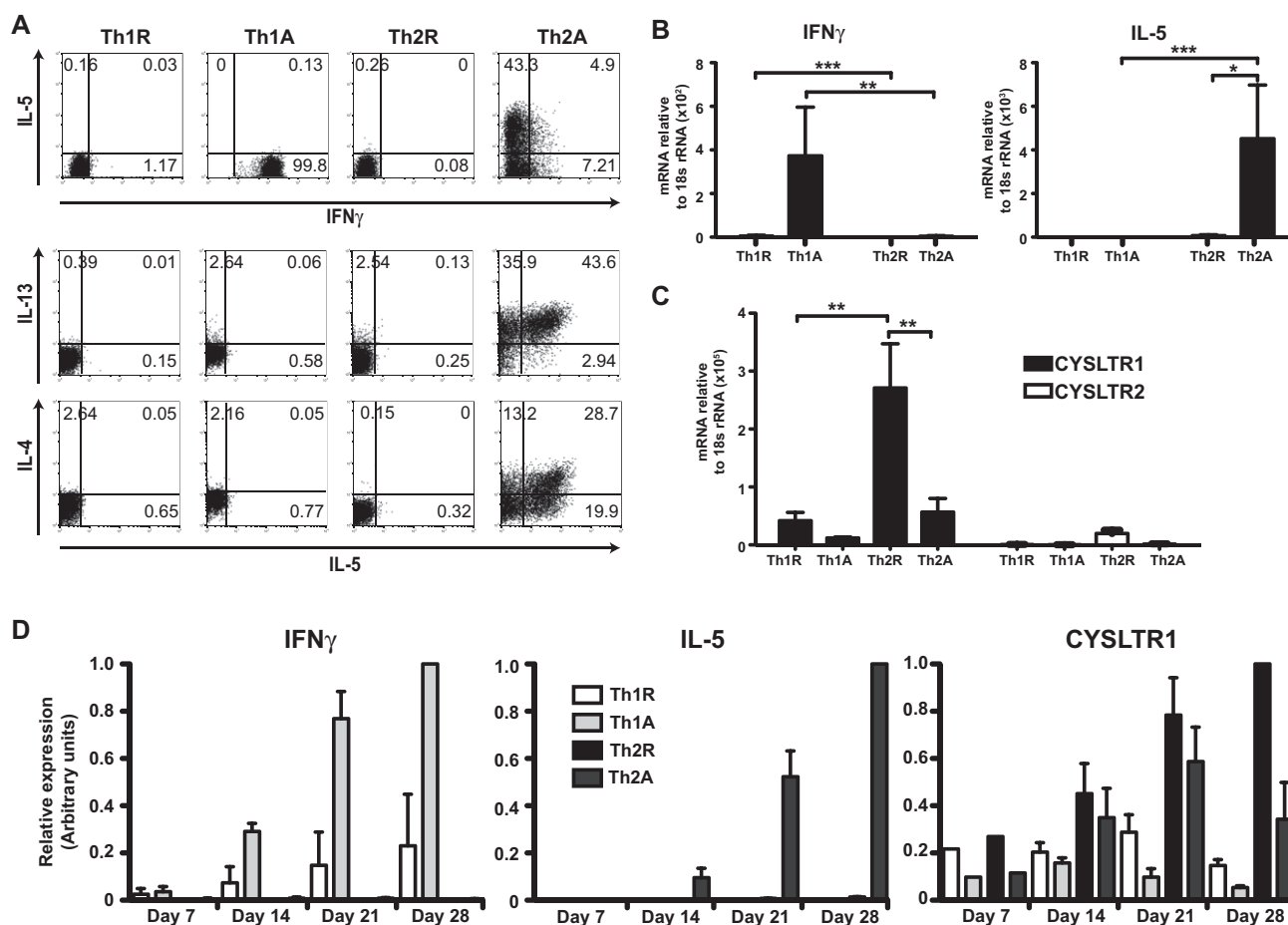


FIG 1. T_H2 cells selectively express CYSLTR1. **A**, Intracellular cytokine staining of human T_H1 and T_H2 cells. Cells were either resting or activated for 4 hours with phorbol 12-myristate 13-acetate (5 ng/mL) and ionomycin (500 ng/mL). **B** and **C**, Real-time RT-PCR analysis of T_H1 and T_H2 cells for IFN- γ and IL-5 (Fig 1, **B**) and CYSLTR1 and CYSLTR2 (Fig 1, **C**). **D**, Time-course analysis of gene expression during T_H1/T_H2 differentiation by using real-time RT-PCR. Data shown are representative of 5 experiments with different donors except Fig 1, **B**, **C**, and **D**, which show means $\pm$ SEMs of 5 experiments. * P < .05, ** P < .01, and *** P < .001, 2-way ANOVA with Bonferroni post test. A, Activated; R, resting.

CysLT-induced calcium flux (Fig 3, **A**). Pertussis toxin partially inhibited calcium mobilization in response to LTD₄, with approximately 50% inhibition of calcium flux at the highest LTD₄ concentration, suggesting that CysLT₁ is coupled to both G α i and G α q G proteins in T_H2 cells. To confirm a role for G α i in CysLT₁ signaling, we examined whether LTD₄ could inhibit cAMP signaling in T_H2 cells. Treatment of T_H2 cells with LTD₄ alone did not induce cAMP in a luminescence-based cAMP assay (Fig 3, **B**). Treatment of T_H2 cells with forskolin induced a robust increase in cAMP levels, which was partially inhibited (approximately 50%) by LTD₄ in a concentration-dependent manner (Fig 3, **B**). Pretreatment of the cells with the CysLT₁ selective antagonist MK571 completely blocked the LTD₄-dependent inhibition of cAMP signaling, confirming that CysLT₁ is partially coupled to G α i in T_H2 cells (Fig 3, **C**). Preincubation of T_H2 cells with thapsigargin to deplete intracellular endoplasmic reticular calcium stores completely inhibited calcium mobilization in response to LTD₄, and chelation of extracellular calcium with EDTA partially inhibited calcium flux (Fig 3, **D**). These data suggest that LTD₄ signaling through CysLT₁ activates store-operated calcium channels in T_H2 cells.

LTD₄ induces chemotaxis of human T_H2 cells

CysLTs have previously been shown to be chemotactic agents for eosinophils, hematopoietic progenitor cells, and monocytes.⁹⁻¹¹ To examine whether LTD₄ treatment causes chemotaxis of human T_H2 cells, we performed a Transwell-based chemotaxis assay.²⁰ The chemokine CXCL12 is strongly chemotactic for human lymphocytes²² and caused a robust chemotactic response in human T_H2 cells (Fig 4). In contrast, CCL18, which has been reported to be selectively chemotactic for human T_H2 cells,²³ did not cause chemotaxis (Fig 4). This might be due to differences in polarization protocols because in the previous study only a single round of *in vitro* polarization was performed. LTD₄ induced concentration-dependent chemotaxis of human T_H2 cells with a classical bell-shaped curve (Fig 4). Maximal chemotaxis was observed at 3 nmol/L LTD₄, which is very similar to the median effective concentration for LTD₄ observed in the calcium mobilization studies.

DISCUSSION

The CysLTs are potent proinflammatory lipid mediators with a major role in the pathogenesis of asthma, including bronchial

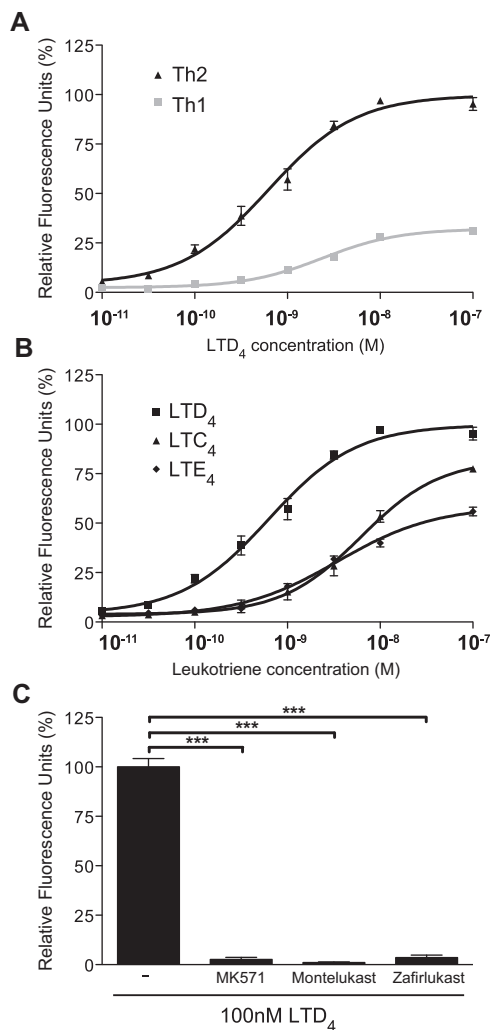


FIG 2. T_H2 cells express functional CysLT₁. **A**, Calcium flux in response to increasing concentrations of LTD₄ in T_H1 and T_H2 cells. **B**, Calcium flux in T_H2 cells in response to increasing concentrations of LTC₄, LTD₄, and LTE₄. **C**, Inhibition of calcium flux in T_H2 cells in response to 100 nmol/L LTD₄ by 3 different CysLT₁ antagonists: MK571, montelukast, and zafirlukast. Data are presented as mean $\pm$ SEM percentages of maximum response to LTD₄ from 3 experiments with different donors. *** P < .001, 1-way ANOVA with Tukey post test.

constriction and cell trafficking. Recent studies have shown that CysLTs also play a critical role in the development and amplification of antigen-specific T_H2 cell-mediated inflammation, although the mechanisms by which this occurs are not fully elucidated.¹² Several studies have implicated myeloid cells in this process, with both dendritic cells^{13,14} and monocytes¹¹ recognized as CysLT target cells. CysLTs have been shown to potentiate dendritic cell functions, such as antigen presentation and cytokine production, in 2 murine models of allergic airway disease.^{13,14} Moreover, the CysLT₁ receptor antagonist pranlukast was shown to inhibit antigen-dependent eosinophilic airway inflammation, mucus production, and airway hyperresponsiveness in an inhalation model of allergic airways disease.¹³ We have previously shown that human monocytes are activated by LTD₄, which induces expression of several immediate early genes and causes chemotaxis.¹¹

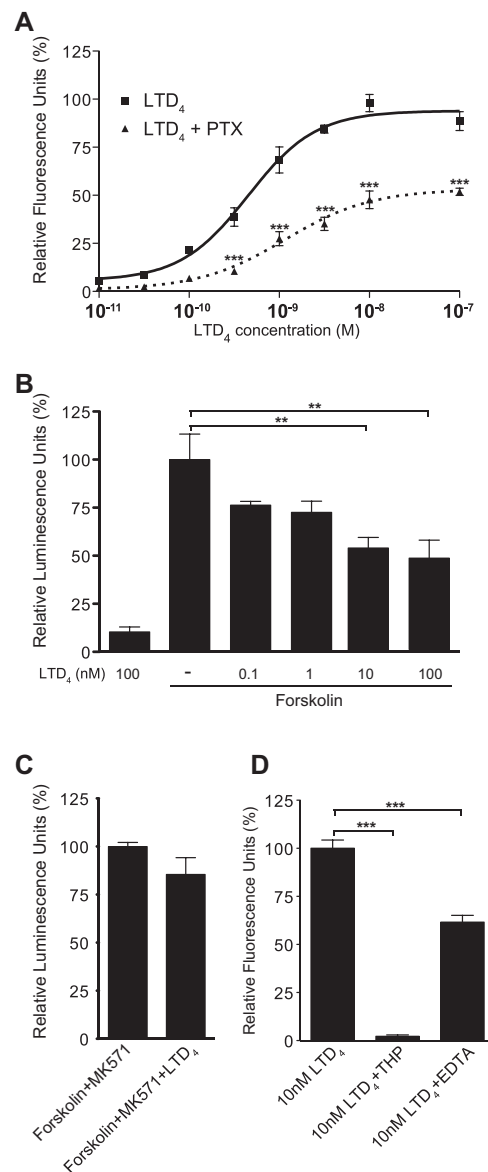


FIG 3. CysLT₁ is partially G_i and G_q coupled in T_H2 cells. **A**, Pertussis toxin (PTX)-mediated inhibition of calcium response to LTD₄ in T_H2 cells. *** P < .001, 2-way ANOVA with Bonferroni post test. **B**, LTD₄-mediated inhibition of cAMP signaling in T_H2 cells. Cells were treated with forskolin to induce cAMP, and increasing concentrations of LTD₄ inhibited cAMP generation. **C**, The CysLT₁ antagonist MK571 blocks the LTD₄-mediated inhibition of cAMP response. Cells were treated with 100 nmol/L LTD₄. **D**, Thapsigargin (THP)-mediated inhibition and EDTA-mediated partial inhibition of calcium flux response to LTD₄ in T_H2 cells. All data are expressed as mean $\pm$ SEM percentages of maximum response to LTD₄ or forskolin from 3 experiments with different donors. Fig 3, B and D: ** P < .01 and *** P < .001, 1-way ANOVA with Tukey post test.

Here we show, for the first time, that human T_H2 cells selectively express *CYSLTR1* mRNA with 6.5-fold higher expression than human T_H1 cells (Fig 1, C). We found no evidence for expression of the other known CysLT receptors, *CYSLTR2*, *GPR17*, or *P2RY12*, in T cells. T_H2 cells selectively responded to CysLTs through calcium signaling in a rank order that resembled, to some extent, previous work on CysLT₁, with LTD₄ being most potent, followed by LTC₄ and LTE₄

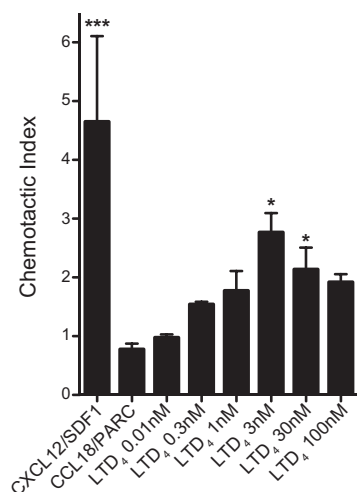


FIG 4. LTD₄ is chemotactic for T_H2 cells. Chemotactic agents were assessed in a Transwell-based system. Cells were allowed to migrate for 2 hours. Varying concentrations of LTD₄ were used as shown. Control chemokines CXCL12 and CCL18 were used at 10 nmol/L concentration. Data shown are means $\pm$ SEMs of 3 independent experiments with different donors. *** $P < .001$ and * $P < .05$, 2-way ANOVA with Bonferroni post test.

(Fig 2, B). Calcium flux in response to LTD₄ was completely inhibited by treatment with the CysLT₁ selective receptor antagonists MK571, montelukast, or zafirlukast (Fig 2, C). These results demonstrate that the selective expression of mRNA for *CYSLTR1* observed in T_H2 cells results in functional CysLT₁ expression. *CYSLTR1* mRNA was significantly downregulated by acute activation of T_H2 cells (Fig 1, C), and activated T_H2 cells were unresponsive to LTD₄ in calcium flux assays (data not shown), suggesting that T-cell activation might cause heterologous desensitization of CysLT₁ in T cells. This is a common feature of several G protein-coupled receptor in T cells, most notably the chemokine receptors, and it suggests that activated T cells have an altered chemotactic potential.²⁴ Treatment of T_H2 cells with the G α_i selective inhibitor pertussis toxin resulted in a 50% inhibition of calcium mobilization, suggesting that CysLT₁ was partially coupled to G α_i in T_H2 cells (Fig 3, A). G α_i G protein-coupled receptors can inhibit increases in intracellular cAMP levels. Treatment of T_H2 cells with LTD₄ partially (50%) inhibited forskolin-induced cAMP generation (Fig 3, B). The LTD₄-dependent inhibition of cAMP signaling could be completely blocked by MK571, demonstrating that it is CysLT₁ dependent (Fig 3, C). Furthermore, LTD₄-induced calcium mobilization is dependent on store-operated calcium channels because it is completely inhibited by thapsigargin (Fig 3, D). Collectively, these data suggest that CysLT₁ is coupled to both G α_i and G α_q G proteins in T_H2 cells. We have also shown that LTD₄ is chemotactic for human T_H2 cells (Fig 4). The peak of migration was observed at 3 nmol/L, with a classical bell-shaped dose-response curve.

A limited number of previous studies have suggested that CysLT₁ might be expressed by T cells.²⁵⁻²⁷ Prinz et al²⁵ showed that murine T cells expressing a mutant version of the linker for activation of T-cells (LAT) adaptor molecule express *Cysltr1* and chemotax in response to LTD₄. Early et al²⁶ showed that short-term treatment with IL-4 caused an increase in *CYSLTR1*

mRNA in T cells. Sharma et al²⁷ recently found evidence for upregulation of *Cysltr1* mRNA in T cells in a compound murine mutant in which the *IL2* gene was deleted in a forkhead box protein 3 mutant strain. However, to our knowledge, the data presented here are the first report of T cell subset-specific expression of any CysLT receptor, and the results strongly suggest that the T_H2 cell should be considered a CysLT target cell. Because LTD₄-mediated effects on T_H2 cells are sensitive to inhibition by the CysLT₁ receptor antagonists MK571, montelukast, and zafirlukast, T_H2 cells might be a previously overlooked target of these clinically efficacious drugs. The importance of CysLTs in the development of T_H2 inflammation in the lung has been demonstrated in murine models of allergic airways disease through both genetic deletion of the LTC₄ synthase gene¹² and pharmacologic blockade with CysLT₁ receptor antagonists.²⁸ Although the mechanisms by which CysLTs function in these systems are unclear, it is tempting to speculate that a direct role in T_H2 cell activation or migration might be involved. Further *in vivo* studies in animal models with conditional deletion of *CYSLTR1* in T cells will be required to fully characterize the relative importance of CysLT₁ expression on T_H2 cell function during asthma pathogenesis.

It has been clear for many years that there are several subphenotypes of allergic asthmatic patients in the population. Since they were first introduced into the clinic, it has been recognized that the CysLT₁ antagonists are effective in only a subset of patients, although no mechanistic explanation of this phenomenon has been identified.²⁹ However, a recent study has suggested that the ratio between urinary LTE₄ and exhaled nitric oxide levels can have some predictive benefit in identifying children who will respond to montelukast.³⁰ Furthermore, novel approaches to endotyping and phenotyping have begun to allow stratification of patients into different groups.³¹⁻³³ A striking example of this is the recent demonstration that asthmatic patients can be stratified into 2 groups based on the level of periostin in the peripheral blood.³⁴ Periostin has been identified as a gene expressed by bronchial epithelial cells in response to IL-13.³³ The authors contend that the periostin level in blood is a clinically useful method to discriminate patients with classical T_H2-driven allergic asthma and patients who do not have such inflammation. In an elegant study they demonstrate that anti-IL-13 might have some benefit in patients with high periostin levels.³⁴ In light of our identification of a role for CysLT₁ in T_H2 cell function, it would be very interesting to examine the effect of CysLT₁ antagonists in a similarly stratified patient group.

In conclusion, we have shown that human T_H2 cells express functional CysLT₁ and that LTD₄ causes chemotaxis of T_H2 cells. These findings suggest a possible explanation for the role of CysLTs in the development or amplification of allergic disease and provide insight into potential mechanisms of action of CysLT receptor antagonists in asthmatic subjects.

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Key messages

- Human T_H2 cells selectively express *CYSLTR1* mRNA.
- Functional analysis shows that T_H2 cells respond to CysLTs.
- LTD₄ is chemotactic for T_H2 cells.

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