

Leukotriene D₄ enhances immunoglobulin production in CD40-activated human B lymphocytes

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Background: Cysteinyl-leukotrienes (cysLTs) are lipid mediators recognized for their role in asthma and allergic inflammation. CysLT₁, one of the receptors for cysLTs, is expressed, among others, in bronchial smooth muscle cells, phagocytes, and B lymphocytes. The potential role of CysLT₁ in B lymphocytes remains unexplored.

Objective: We examined the modulation of expression and function of CysLT₁ in human B lymphocytes.

Methods: Human B lymphocytes were purified from peripheral blood and analyzed by means of RT-PCR and flow cytometry, after culture with IL-4 and anti-CD40 antibody or CD154-transfected fibroblasts. Functional responses to leukotriene (LT) D₄ in terms of cytosolic calcium flux, proliferation, and immunoglobulin production were also examined.

Results: B lymphocytes expressed CysLT₁ at both the mRNA and protein levels. Two-fold to 3-fold enhancement of CysLT₁ expression was observed after B-cell exposure to a combination of activating anti-CD40 antibody and IL-4. The expression of CysLT₁ was also enhanced when B lymphocytes were cocultured with CD154-transfected fibroblasts in the presence of IL-4. Moreover, IL-4 and CD40-activated B lymphocytes showed an increased responsiveness to LTD₄ in terms of cytosolic calcium flux, which was totally prevented by the selective CysLT₁ antagonist montelukast. Stimulation of IL-4 and CD40-activated B lymphocytes with picomolar concentrations of LTD₄ induced mature epsilon transcripts and upregulated IgE and IgG production 2-fold to 3-fold.

Conclusion: We have demonstrated that expression of CysLT₁ can be upregulated in B lymphocytes after stimulation with CD154, with consequent increased responsiveness of the cells to LTD₄ in terms of immunoglobulin production.

Clinical implications: This study provides evidence that cysLTs generated during allergic reactions can affect B-cell function

and enhance IgE and IgG production, which may further contribute to the allergic process. (*J Allergy Clin Immunol* 2006;117:924-30.)

Key words: B cells, lipid mediators, antibodies, cell surface molecules, receptor, IL-4

The cysteinyl-leukotrienes (cysLTs) leukotriene (LT) C₄, LTD₄, and LTE₄ are lipid mediators well known for their proinflammatory properties, particularly in the pathology of asthma. They are produced by eosinophils, basophils, mast cells, alveolar macrophages, and monocytes through the metabolism of arachidonic acid via the 5-lipoxygenase pathway,¹ after cellular activation through various stimuli.² CysLTs are recognized as very potent bronchoconstrictors, although their biological effects are wider than initially expected. These effects include several actions relevant to the etiopathogenesis of asthma such as airway smooth muscle contraction, vasoconstriction, or endothelium-dependent relaxation and eosinophil recruitment.² CysLTs also affect the inflammatory response by inducing the production of cytokines required to stimulate T-cell responses or determining the homing of dendritic cells.^{3,4} Whereas pharmacologic profiling studies suggested the presence of at least three receptors for cysLTs,^{3,5,6} 2 receptors for cysLTs (CysLT₁ and CysLT₂) have been cloned and characterized.⁷⁻¹⁴ Human CysLT₁ and CysLT₂ cDNAs encode proteins of 337 and 347 amino acids, respectively, with 7 hydrophobic, putatively transmembrane, domains linked by 6 hydrophilic loops, typical of G-protein-coupled receptors. CysLT₂ is expressed ubiquitously,¹¹⁻¹³ whereas CysLT₁ is expressed on bronchial smooth muscle cells, monocytes, macrophages, eosinophils, neutrophils, intestinal epithelial cells, mast cells, and B lymphocytes.^{7,8,15-19} Binding affinities of cysLTs have been characterized for each receptor, but there is no selective ligand for either receptor. CysLT₁ binds preferentially LTD₄, followed by LTC₄ and LTE₄,⁷⁻¹⁰ whereas LTD₄ and LTC₄ have equal affinities for CysLT₂.¹¹⁻¹⁴ We and others have shown that CysLT₁ expression can be modulated in monocytes, eosinophils, and bronchial smooth muscle cells. In the presence of cytokines such as IL-5, IL-13, IL-4, or TGF-β, expression of CysLT₁ can be upregulated at the RNA and protein levels in these different cellular models.^{16,20-23} The signaling pathways involved in the modulation of CysLT₁ expression have not yet been elucidated, and characterization of the CysLT₁ gene promoter will

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Abbreviations used

CysLT: Cysteinyl leukotriene
L4.5: CD154-transfected murine L929 fibroblasts
LT: Leukotriene
NF- κ B: Nuclear factor- κ B
PAF: Platelet-activating factor
PBL: Peripheral blood lymphocyte
PMA: Phorbol 12-myristate 13-acetate
STAT: Signal transducer and activator of transcription

eventually help define the transcription factors involved in this pathway.

As producers of antibodies, B lymphocytes play a pivotal role in the immune response. This role is greatly dependent on the *in vivo* environmental conditions such as presence of growth factors, cytokines, and intercellular contacts.²⁴ Several signals are required for maximal B-cell activation, including interactions of MHC molecules with the T-cell receptor as well as other costimulatory signals such as CD40 with CD154 and CD28 with CD80/CD86. Indeed, these signals influence B-cell activation and proliferation, as well as cellular differentiation and production of immunoglobulins.²⁵ CD40, a member of the superfamily of TNF/TNF receptor, is largely involved in the activation, proliferation, and differentiation of B lymphocytes. CD40 is essential for isotype class switching, but the transduced signal can be modulated by the various cytokines present during the interaction with CD40.^{26,27} The signaling pathway of CD40 leads to activation of several transcription factors including nuclear factor- κ B (NF- κ B), activator protein-1, and signal transducer and activator of transcription (STAT),²⁸⁻³⁴ with subsequent gene transcription of proteins such as CD54, CD80/CD86, and CD23.^{35,36}

Until now, however, the effects of cysLTs and their receptors on B-lymphocyte function have not been reported. Because CysLT₁ expression can be upregulated in other leukocyte populations by cytokines, with subsequently increased responsiveness of the cells to cysLTs, we speculated that the expression of the CysLT₁ receptor could be modulated in human B lymphocytes by interaction between CD40 and its ligand, CD154, in the presence of IL-4. Unstimulated and stimulated B lymphocytes were therefore examined for CysLT₁ expression as well as functional responses to LTD₄ in terms of cytosolic calcium flux and immunoglobulin production.

METHODS

Reagents and antibodies

IL-4, TNF- α and IFN- γ were obtained from Peprotech (Ottawa, Ontario, Canada). IL-13 was obtained from R&D Systems (Minneapolis, Minn). Rabbit antihuman CysLT₁ Abs were obtained from Cayman Chemical (Ann Harbor, Mich) and activating anti-CD40 (G28.5) Abs from Dr Walid Mourad (Université Laval, Quebec, Canada). Platelet-activating factor (PAF) and LTD₄

were obtained from Cayman Chemical and phorbol 12-myristate 13-acetate (PMA) from Sigma (Oakville, Ontario, Canada).

Cell culture

Peripheral blood lymphocytes (PBLs) were obtained from healthy volunteer donors, after approval by our Institutional Review Board and after informed consent from volunteers, and purified by using Ficoll-Hypaque (Amersham-Pharmacia, Baie d'Urfé, Quebec, Canada) centrifugation and depletion of monocytes by adhesion to plastic dishes, as described by Thivierge et al.¹⁶ B lymphocytes were then isolated by CD19 MicroBeads using the Miltenyi technology (Miltenyi Biotecnologies, Auburn, Calif). Stimulation of PBLs was performed during a 72-hour culture by using G28.5 (5 μ g/mL), IL-4 (10 ng/mL), PMA (10^{-7} mol/L), TNF- α (20 ng/mL), IFN- γ (100 U/mL), or PAF (10^{-7} mol/L) in Iscove's medium supplemented with 10 % heat-inactivated FBS (Sigma).

Peripheral blood lymphocytes and B cells were also cultured in microtiter plates with irradiated CD154-transfected murine fibroblasts (L4.5; gift from Dr André Darveau, Université Laval, Quebec, Canada) in Iscove's medium supplemented with 10% FBS, 5 μ g/mL human transferrin (Sigma), 5 μ g/mL bovine insulin (Sigma), 100 U/mL human IL-4, 50 U/mL penicillin, and 50 μ g/mL streptomycin as described.^{37,38} Half of the medium was replaced every 3 days and the L4.5 cells every 6 days.

Measurement of CysLT₁ receptor expression

Cells were harvested, fixed in 2% paraformaldehyde, and permeabilized with 0.1% saponin. After blocking with human IgG (Sigma), rabbit antihuman CysLT₁Ab was used in combination with biotin-conjugated goat antirabbit IgG and streptavidin-Cy5 (Jackson ImmunoResearch Laboratories, West Grove, Pa) to assess CysLT₁ expression. B cells were identified with fluorescein isothiocyanate-conjugated mouse anti-CD19 Ab (BD BioSciences, Mississauga, Ontario, Canada). Cells were analyzed with a FACScalibur flow cytometer and CellQuest Pro software (BD BioSciences).

RT-PCR

CysLT₁ RNA was purified by using Tripure reagent according to the manufacturer's instructions (Roche, Laval, Quebec, Canada). After treatment with DNase I (Ambion, Austin, Tex), cDNA was synthesized with oligo dT (Amersham-Pharmacia) and reverse transcriptase (M-MLV; BioCan, Mississauga, Ontario, Canada). PCR was then performed by using Taq polymerase (New England Biolabs, Pickering, Ontario, Canada) and oligos specific for CysLT₁ for 35 cycles and an annealing temperature of 62°C as described by Espinosa et al.²³ Quantitative controls were performed according to the same procedure as for CysLT₁ and using specific oligos for human glyceraldehyde phosphate dehydrogenase.²³ Densitometry quantification was performed by using the ImageJ 1.32 software (National Institutes of Health, Bethesda, Md).

Mature epsilon transcript. RNA was purified and cDNA was synthesized as described. PCR was performed as described by Cerutti et al.³⁹ by using oligos specific for productive transcript of epsilon for 30 cycles and an annealing temperature of 60°C. Quantitative controls were also performed by using specific oligos for hGAPDH for 25 cycles. Densitometry was measured by using the ImageJ 1.32 software.

Cytosolic calcium mobilization assay

Real-time mobilization by cytometry. Cells were loaded with 3 μ mol/L Fluo-3 (Molecular Probes, Eugene, Ore) for 30 minutes at room temperature. After washing, LTD₄ (10^{-7} mol/L) was added to cells, pretreated or not with montelukast (Merck-Frosst, Pointe-Claire, Quebec, Canada), a selective cysLT₁ antagonist. Analysis was performed by cytometry using FACScan (BD BioSciences).

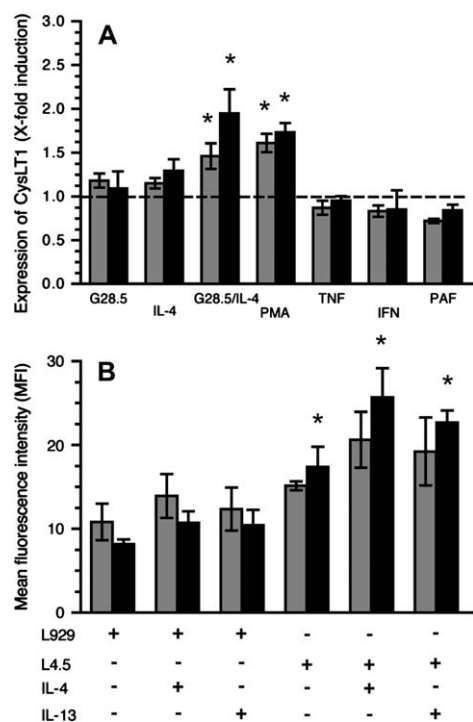


FIG 1. A, Modulation of expression of CysLT₁ in CD19⁺ cells from PBLs by anti-CD40 Ab (G28.5; 5 μ g/mL), IL-4 (10 ng/mL), PMA (10⁻⁷ mol/L), TNF- α (20 ng/mL), IFN- γ (100 U/mL) or PAF (10⁻⁷ mol/L). PBLs were stimulated for 48 (gray bars) and 72 (black bars) hours before analysis by flow cytometry. B, Expression of CysLT₁ in purified CD19⁺ B lymphocytes. Cells were stimulated by coculture with irradiated murine L929 fibroblasts or with CD154-transfected L929 fibroblasts (L4.5) in the absence or presence of IL-4 (100 U/mL) or IL-13 (20 ng/mL) for 48 (gray bars) and 72 (black bars) hours before analysis by flow cytometry. Geometrical mean fluorescence intensity $\pm$ SEM is presented; n = 3-7; *P < .05.

Spectrophotometric measurement of calcium mobilization. Cells were loaded with 3 μ M Fura-2 (Molecular Probes) for 15 minutes at room temperature. After washing, LTD₄ (10⁻⁷ mol/L) was added to cells, pretreated or not with 10⁻⁷ mol/L montelukast. Analysis was performed by spectrophotometry using the Hitachi (Tokyo, Japan) F2500 fluorescence spectrophotometer and FL solutions software (version 1.0).

Measurement of immunoglobulin secretion

Cell culture. B lymphocytes were seeded at 5×10^3 cells/well in a 96-well microplate by using the CD154 system as described in presence or absence of stimuli, as indicated. After 14 days of culture, supernatants were harvested and frozen until quantification.

Quantification of IgG, IgM, and IgE. Microplates (Costar, Corning, NY) were coated with 5 μ g/mL goat antihuman IgG, IgM (Sigma), or IgE (Chemicon, Temecula, Calif). After incubation at 4°C overnight, wells were blocked with PBS, 0.05% Tween 20, 5% FBS and washed with PBS, 0.05% Tween 20 (BioRad, Mississauga, Ontario, Canada). Samples or standards (human IgG or IgM; Sigma; or human IgE; Calbiochem, Mississauga, Ontario, Canada) diluted with PBS, 0.05% Tween 20, 1% FBS were distributed in duplicate into wells and plates were incubated for 60 minutes at 37°C. Bound antibodies were detected by using alkaline phosphatase-labeled goat antihuman IgG, IgM, or IgE (Sigma) and paranitrophenol phosphate (Sigma). Optical densities were read at 405 nm with a reference wavelength of 630 nm.

Determination of immunoglobulin-secreting populations (ELISPOT). B lymphocytes were cultured in the CD154 system as described in presence of graded concentrations of LTD₄. Cells were deposited for 6 hours at 37°C on high-affinity binding microtiter plates (Costar) precoated with goat antihuman IgG, IgM, or IgE (10 μ g/mL). Immunoglobulin-producing cells were detected by using alkaline phosphatase-labeled goat-antihuman IgG, IgM, or IgE and BCIP (Sigma). Spots were counted under microscopy by using 100 $\times$ magnification.

Statistical analysis

Results were expressed as means $\pm$ SEMs. The differences between group means were analyzed by using 1-way ANOVA. Statistical significance is indicated by asterisks for P < .05.

RESULTS

Expression of CysLT₁ in B lymphocytes

Freshly isolated human B lymphocytes expressed low levels of CysLT₁ (mean fluorescence intensity of unstimulated cells: 9.1 ± 1.5 ; isotypic control: 5.9 ± 2.0). Their stimulation with an anti-CD40 (G28.5) antibody or with IL-4 alone did not significantly enhance the expression of CysLT₁, whereas a combination of these 2 agents induced as much as a 2-fold increase in expression of CysLT₁ (Fig 1, A). PMA, a protein kinase C activator and a powerful mitogen recognized to induce activation and differentiation of B cells,^{40,41} also augmented the expression of CysLT₁, but to a lesser extent than the combination of anti-CD40 Ab and IL-4. Blocking protein kinase C activity with GF109203X, however, did not prevent anti-CD40 Ab and IL-4-induced CysLT₁ expression. TNF- α , IFN- γ , and PAF did not affect the expression of CysLT₁ (Fig 1, A). On the other hand, CysLT₂ expression remained constant during stimulation (data not shown). These results indicated that some, but not all, stimuli that can induce the activation of B lymphocytes are also capable of upregulating their expression of CysLT₁.

Stimulation of B lymphocytes with irradiated CD154-transfected murine fibroblasts (L4.5) in combination with IL-4 allows B lymphocytes to be activated, to differentiate, and to produce immunoglobulins. This system mimics the *in vivo* environment and the stimulation within germinal centers.^{26,37} We used this system to stimulate purified CD19⁺ B lymphocytes during a 72-hour time-course (Fig 1, B). The combination of L4.5 and IL-4 or IL-13 induced an optimal upregulation of CysLT₁ expression, similar to that observed with anti-CD40 Ab (G28.5) and IL-4. At 72 hours of stimulation, CysLT₁ expression was increased as much as 3-fold over the basal level of unstimulated B cells. Untransfected L929 fibroblasts in the presence or absence of IL-4 or IL-13 did not enhance CysLT₁ expression, whereas irradiated L4.5 cells, alone, slightly augmented the expression of CysLT₁ in B lymphocytes. Levels of expression of the CysLT₁ receptor reached a plateau after 3 days of culture and remained elevated for as long as 21 days of culture (data not shown).

Semiquantitative RT-PCR revealed that the steady-state levels of CysLT₁ mRNA were also upregulated in B lymphocytes stimulated through the CD154 system (Fig 2, A).

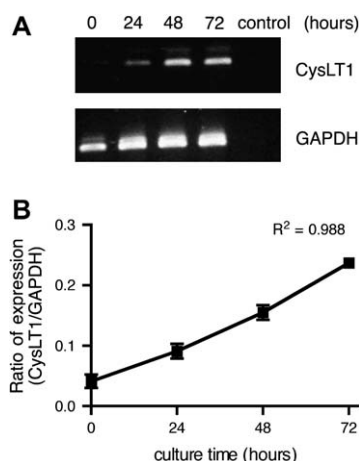


FIG 2. Modulation of expression of CysLT₁ in purified B lymphocytes stimulated in the CD154 system. Cells were stimulated through a 72-hour time course and harvested at indicated times. Steady state levels of CysLT₁ and glyceraldehyde phosphate dehydrogenase mRNA were measured by semiquantitative RT-PCR (**A**) and further quantified by densitometry (**B**). Control lane was PCR reaction in absence of RT. *n* = 3.

Densitometry quantification showed a proportional increase of CysLT₁ expression as a function of time of stimulation during the initial 72 hours (Fig 2, *B*), indicating that CD40 activation led to the upregulation of CysLT₁ in terms of both mRNA and protein expression. This increase was stable over time, reaching a plateau of mRNA expression at day 3 and lasting until day 9 (data not shown).

Activity of CysLT₁

To determine the level of responsiveness of B lymphocytes to LTD₄, we performed cytosolic calcium flux experiments. Calcium mobilization was compared between unstimulated and CD40-activated B lymphocytes. Whereas unstimulated cells did not respond to LTD₄, cells stimulated with IL-4 or L4.5 alone showed a discrete response, and the combination of these 2 signals resulted in an enhanced response to the ligand (Fig 3, *A*). These results suggest that basal expression of CysLT₁ on B lymphocytes is not sufficient to produce a significant responsiveness to LTD₄, but that the upregulated expression of CysLT₁ on activated B lymphocytes enables them to increase their cytosolic calcium concentration when stimulated with LTD₄. In another set of experiments, to assess whether LTD₄ induced cytosolic calcium flux through interaction with CysLT₁, we used montelukast, a selective CysLT₁ antagonist. B lymphocytes were activated during 5 days in the CD154 system before analysis of their calcium mobilization response to LTD₄. Pretreatment of CD40-activated B lymphocytes with montelukast totally prevented the cytosolic calcium flux after LTD₄ stimulation (Fig 3, *B*). This was also obvious at the single cell level, using confocal microscopy (data not shown). Montelukast alone had no effect on calcium mobilization in B cells (data not shown). These data indicated that stimulation of CD40-activated B lymphocytes

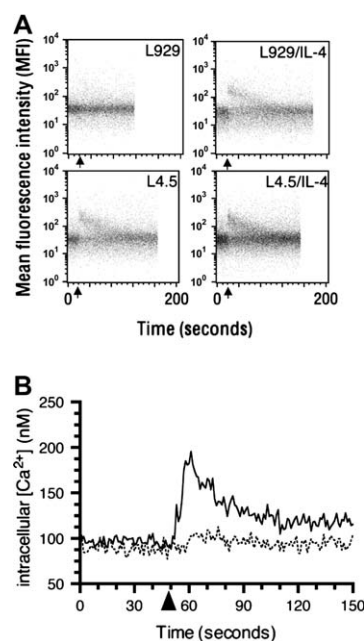


FIG 3. **A**, Calcium mobilization in CD40-activated B lymphocytes. Cells were stimulated for 72 hours in presence or absence of IL-4 in combination with untransfected (L929) or CD154-transfected (L4.5) fibroblasts. Cells were then loaded with Fluo-3 before stimulation with 10⁻⁷ mol/L LTD₄ and analysis by flow cytometry. **B**, Cells were stimulated with 10⁻⁷ mol/L LTD₄ in the absence (*plain line*) or presence of pretreatment with 10⁻⁷ mol/L montelukast (*dotted line*). Arrows indicate the time of leukotriene addition. These graphics are representative of 3 to 5 separate experiments.

with LTD₄ involved signaling mediated by CysLT₁ rather than CysLT₂.

Modulation of immunoglobulin production by CysLT₁

Because of the involvement of cysLTs in asthma and allergic inflammation, we speculated that cysLTs might also be able to affect immunoglobulin isotype switching toward IgE. When B lymphocytes were stimulated in the CD154 system in the absence or presence of graded concentrations of LTD₄, enhanced mature epsilon transcript expression was observed, with optimal expression at 10 pmol/L LTD₄ (Fig 4). Montelukast (10⁻⁷ mol/L) totally prevented the enhanced expression of epsilon transcripts induced by LTD₄ (data not shown).

To determine the effect of LTD₄ on the production of immunoglobulins, B lymphocytes were stimulated in the CD154 system in the absence or presence of graded concentrations of LTD₄. ELISPOT assays were performed to evaluate the populations of IgG, IgM, and IgE secretors, and ELISAs were used to assess the overall production of IgG, IgM, and IgE by the whole population of CD40-activated B lymphocytes. Picomolar concentrations of LTD₄ were most effective in augmenting the numbers of IgE secretors 2-fold to 3-fold (Fig 5, *A*). The numbers of IgM secretors were also augmented by LTD₄, whereas IgG secretors were less affected (Fig 5, *B and C*). These

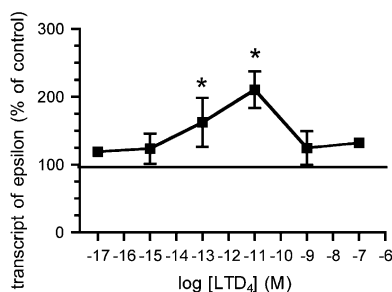


FIG 4. Expression of mature epsilon transcripts in CD40-activated B lymphocytes exposed to graded concentrations of LTD₄. Cells were stimulated as described for 14 days, and RNA expression of mature epsilon transcript and GAPDH control gene was measured in semi-quantitative RT-PCR. Densitometry was used to calculate the epsilon/GAPDH ratio, which was normalized to 100% for control, LTD₄-unstimulated cultures. Each treatment was then compared with the LTD₄-unstimulated condition. Data are means $\pm$ SEMs; n = 2-7; *P < .05.

results suggest that LTD₄ can enhance the numbers of CD40-activated B lymphocytes that produce IgM and IgE.

Overall production of immunoglobulins was also enhanced by LTD₄ in a concentration-dependent manner. Total IgE production was augmented as much as 2-fold by picomolar concentrations of LTD₄ (Fig 5, D). Interestingly, total IgM production was not significantly affected, whereas IgG production was enhanced 2-fold (Fig 5, E and F). These results indicate that LTD₄ can significantly upregulate the production of IgG and IgE in CD40-stimulated B lymphocytes.

DISCUSSION

Responses of airways and lung parenchyma to cysLTs have long been recognized,^{2,42} and CysLT₁ expression has been found initially in bronchial smooth muscle cells and in alveolar macrophages.⁷ More recently, CysLT₁ expression was also reported in human B lymphocytes,¹⁵ but its potential role in this cell population has not been defined. In this study, we show for the first time that CysLT₁ expression can be modulated by biological stimuli in human B lymphocytes, thus rendering the cells responsive to stimulation by LTD₄. Indeed, human B lymphocytes activated through their CD40 receptor in conjunction with IL-4 show an upregulation of CysLT₁ expression with subsequent responsiveness to LTD₄ in terms of cytosolic calcium flux and secretion of immunoglobulins. We have previously shown that IL-4 and IL-13 can induce the upregulation of CysLT₁ expression in human monocytes and macrophages, which leads to enhanced chemotactic responses to LTD₄.¹⁶ In contrast, IL-4 or IL-13 alone was not as effective in upregulating CysLT₁ expression in human B cells, but it could synergize with CD40-mediated activation for maximal receptor expression.

The upregulation of CysLT₁ expression on human CD40-activated B lymphocytes suggested that stimulation

with leukotrienes could affect the functions of B cells. Mobilization of intracellular calcium was used as the functional short-term read-out of CysLT₁ activation in B cells. Although unstimulated B cells expressed few receptors for LTD₄ and did not respond to LTD₄, CD40-activated B cells showed upregulated CysLT₁ expression and could be stimulated by LTD₄, with consequent induction of cytosolic calcium flux. These observations demonstrate the biological functionality of the upregulated CysLT₁ on CD40-activated B cells. Furthermore, a selective antagonist for CysLT₁, montelukast, could completely prevent this biological effect, suggesting that the mobilization of cytosolic calcium after stimulation with LTD₄ in CD40-activated human B lymphocytes involved the CysLT₁ receptor. Stimulation with LTD₄ did not significantly affect B-cell proliferation (data not shown), however, indicating that CysLT₁ did not modulate the cell cycle in these cells. In contrast, LTB₄, which is also a lipid mediator recognized for its immunomodulatory properties, has been shown to activate B cells and enhance their proliferation as well as the production of immunoglobulin.⁴³⁻⁴⁵

The production of immunoglobulin was also studied in response to LTD₄ in CD40-activated B lymphocytes. Indeed, this stimulation revealed significant increases in IgE and IgG secretion, whereas IgM production was essentially unaffected. Both higher numbers of IgE producers and higher total production of IgE were induced by picomolar concentrations of LTD₄. In contrast, whereas total IgG secretion was augmented by LTD₄, numbers of IgG producers were only marginally increased, but the IgG production spots in the ELISPOT assay were larger at picomolar and nanomolar concentrations of LTD₄, corresponding to the increased production of IgG. Conversely, numbers of IgM producers were increased by LTD₄, but their IgM production spots were smaller in size, corresponding to a lack of augmented overall production of IgM by LTD₄.

The source of cysLTs, which would interact and affect B-cell function, remains to be identified. However, antigen-presenting cells such as macrophages and dendritic cells are known to produce cysLTs.^{1,2,46} In the environment of the spleen, the lymph nodes and, quite plausibly, the bronchus-associated lymphoid tissue (BALT),⁴⁷ dendritic cells migrating from inflammatory sites may well provide cysLTs to the CD40-activated B lymphocytes and thus affect their immunoglobulin production. Moreover, mast cells migrating into lymph nodes would provide a rich source of cysLTs.⁴⁸ Finally, production of cysLTs by eosinophils, basophils, mast cells, and macrophages of the inflamed lung could provide cysLTs through diffusion into the B-cell compartment of bronchus-associated lymphoid tissue.

Several signaling pathways may be involved in the upregulation of CysLT₁ in CD40-activated B cells. CD40 signaling involves the activation of NF- κ B as well as the JAK3-STAT and mitogen-activated protein kinase pathways.²⁷⁻³⁴ Transgenic models have shown that the absence of an active complex of NF- κ B or the JAK3 protein impairs greatly the cellular activation through

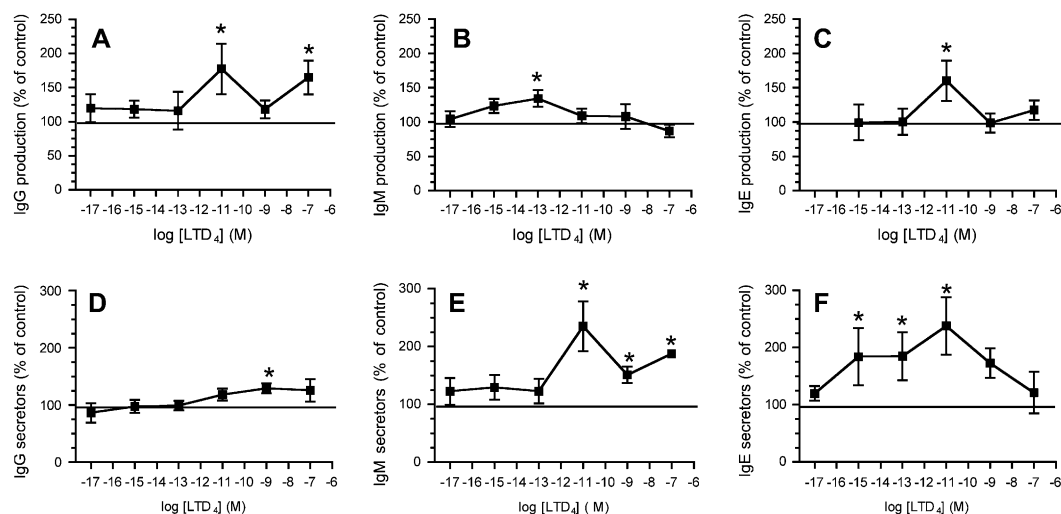


FIG 5. Production of immunoglobulins by IL-4 and CD40-activated B lymphocytes after treatment with graded concentrations of LTD₄. *Upper panels*, Secretion of IgG (A), IgM (B), and IgE (C) was measured by ELISA in supernatants of B cells after 14 days of costimulation with IL-4, CD154-transfected fibroblasts, and graded concentrations of LTD₄. *Lower panels*, Producers of IgG (D), IgM (E), and IgE (F) were quantified by ELISPOT using B cells after 14 days of costimulation with IL-4, CD154-transfected fibroblasts, and graded concentrations of LTD₄. The production level of each treatment was then compared with the LTD₄-unstimulated condition, normalized to 100%. Data are means $\pm$ SEMs; n = 3-10; *P < .05.

CD40.^{34,49-51} These pathways could participate in the CD40 signaling, leading to enhanced CysLT₁ expression. Moreover, IL-4 is recognized as an activator of STAT6,⁵² and our previous findings suggest that STAT6 may be involved in the transactivation of the CysLT₁ promoter in phagocytes, because both IL-4 and IL-13 strongly up-regulate CysLT₁ expression in these cells.^{16,22} We thus propose that NF- κ B and STAT6 synergize in the upregulation of expression of the CysLT₁ receptor in B lymphocytes. These pathways are currently being investigated in our laboratory to determine the transactivation mechanisms of the CysLT₁ gene in human B lymphocytes.

In conclusion, we have reported that B cells respond to IL-4 and CD40-mediated costimulation by upregulating the expression of CysLT₁ and subsequently becoming responsive to cysLTs. CysLTs can then induce activated B cells to produce greater quantities of IgE and IgG. These findings shed new light on the interactions of cysLTs with the adaptive immune response and may provide a new link between these lipid mediators and the development or persistence of atopic inflammation and asthma.

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