

Role of the LTB₄/BLT1 Pathway in Allergen-induced Airway Hyperresponsiveness and Inflammation

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ABSTRACT

LTB₄, a proinflammatory lipid mediator generated from arachidonic acid through the action of 5-lipoxygenase, has been known for over two decades and is implicated in a wide variety of inflammatory disorders. BLT1, a G-protein-coupled receptor, has recently been identified as a high affinity receptor specific for LTB₄. Recent studies in allergen-induced airway hyperresponsiveness and inflammation using mice lacking BLT1 have shown crucial new roles for leukotriene B₄ and BLT1 in Th2 cytokine IL-13 production from lung T cells and recruitment of antigen-specific effector CD8⁺ T cells, suggesting novel mechanisms for their actions. The leukotriene B₄-BLT1 pathway is an important target for the treatment of bronchial asthma.

KEY WORDS

airway responsiveness, cytokines, lipid mediators, lung inflammation, T cells

INTRODUCTION

Allergic asthma is a complex syndrome which has been characterized by airway obstruction, airway inflammation and airway hyperresponsiveness (AHR).^{1,2} Allergen-specific memory T cells and antibody are thought to play a central role in the development of these responses.^{2,3} Recent studies in both humans and rodents have indicated that in addition to CD4⁺ T cells, CD8⁺ T cells may play an important role in the development of allergic airway responses.⁴⁻¹⁰

Leukotriene B₄ (LTB₄) is a potent lipid inflammatory mediator derived from membrane phospholipids by the sequential action of cytosolic phospholipase A₂, 5-lipoxygenase and leukotriene A₄ hydrolase.^{11,12} LTB₄ is a chemoattractant for leukocytes, including neutrophils, macrophages, monocytes, and eosinophils.¹³⁻¹⁶ LTB₄ has classically been thought to be an important mediator of the early phase of the asthmatic response to inhaled allergens.¹⁷ Recently, the receptors for LTB₄ have been identified, and LTB₄ is thought to activate leukocytes through a G protein-coupled cell surface receptor, BLT1.^{18,19} As we dis-

cuss here, the discovery of BLT1 and recent experiments using mice that are deficient in BLT1 have revealed important roles for this LTB₄-BLT1 pathway in IL-13 production from lung T lymphocytes and recruitment of effector CD8⁺ T cells in allergen-induced airway hyperresponsiveness and inflammation.

GENERATION OF LTB₄

Leukotrienes are generated by the metabolism of arachidonic acid. The first committed step in the leukotriene biosynthesis is conversion of arachidonic acid to leukotriene A₄ (LTA₄) by the enzyme 5-lipoxygenase (5-LO).¹¹ Leukotrienes do not exist preformed in cells but are synthesized from the breakdown of arachidonic acid. They can be released from phospholipids in intact cells following stimulation, infection or injury by the action of cytosolic phospholipase A₂. The free arachidonic acid is sequestered at the nuclear envelope and brought into contact with 5-LO by an accessory protein named 5-LO-activating protein (FLAP). 5-LO converts arachidonic acid to LTA₄, which is unstable and is hydrolyzed by LTA₄ hydrolase (LTA₄H), to form LTB₄.

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The leukotrienes are formed in different cell types as well as via transcellular metabolism involving multiple cells such as neutrophils, platelets, and vascular cells.²⁰⁻²² Human neutrophils and eosinophils synthesize LTB₄ and LTC₄, respectively.^{13,23} Monocytes and macrophages also synthesize both LTB₄ and the cys-LTs.²⁴ LTC₄ is metabolized to LTD₄ and LTE₄ by the cells in which this mediator is formed. In addition, the cys-LTs can be transformed into 6-*trans*-LTB₄ by hypochlorous acid, which is generated during the respiratory burst in leukocytes.^{25,26} LTB₄ is also metabolized in the cells which produce this metabolite, by a unique membrane bound cytochrome P 450 enzyme.²⁷

RECEPTORS FOR LTB₄

Although LTB₄ was one of the earliest leukocyte chemoattractants identified, its receptors have been elusive. The human high-affinity receptor was cloned by Yokomizo *et al.* in 1997 from differentiated HL-60 cells and provided a molecular tag for LTB₄ activities.¹⁸ A second, low-affinity LTB₄ receptor, BLT2 was also identified by Yokomizo *et al.*¹⁹ Both receptors are members of the G-protein-coupled seven-transmembrane-domain receptor superfamily, whose genes are located in very close proximity to each other in the human as well as mouse genomes. The two receptors differ in their affinity and specificity for LTB₄. BLT1 is a high affinity receptor specific for LTB₄, whereas BLT2 is a low affinity receptor which also binds other eicosanoids. These two receptors also differ in their expression pattern: BLT1 is expressed primarily on leukocytes, whereas BLT2 is expressed more ubiquitously.¹⁶

LTB₄ was classically described as a chemoattractant for myeloid leukocytes,^{13,28} and BLT1 was shown to be expressed on granulocytes, macrophages, monocytes and eosinophils, and to a lesser extent on naive lymphocytes.^{15,16,29-32} These findings were consistent with the classical notion that LTB₄ is a local inflammatory mediator. However, recent studies have suggested that it also acts as an important attractant for differentiated T cells. Subsequently, BLT1 expression on mouse CD4⁺ T cells³³ that have been differentiated *in vitro* to effector phenotypes has been reported. CD4⁺ T cells that were activated *in vitro* under non-polarizing (TH0), TH1-polarizing, or TH2-polarizing conditions all had increased levels of mRNA encoding BLT1 compared with naive cells, which expressed little BLT1.³³ By contrast, expression of BLT2 by naive T cells or by TH0, TH1 or TH2 effector cells was not detected. BLT1 expression has also been shown to be induced in CD4⁺ T cells that leave the lymph node and enter the tissue after activation by antigen *in vivo* in intact mice.³³

BLT1 expression on mouse CD8⁺ T cells that have been differentiated *in vitro* to effector phenotypes has also been reported.^{34,35} Antigen-experienced popula-

tions of memory CD8⁺ T cells can be distinguished by the surface expression of CD62 ligand (CD62L) and the chemokine receptor CCR7. Different functional and migratory properties have been recently ascribed to these cells.³⁶⁻³⁸ Antigen-experienced central memory CD8⁺ T cells (T_{CM}) are CD62L^{hi}/CCR7^{hi} and home preferentially to lymph nodes. Effector CD8⁺ T cells (T_{EFF}) are CD62L^{lo}/CCR7^{lo} and traffic more efficiently to nonlymphoid tissues and to sites of tissue inflammation. *In vitro*, CD8⁺ T cells can be differentiated in culture to either of these subtypes. When cultured in the presence of IL-15, antigen-specific CD8⁺ T cells acquire the phenotypic and functional characteristics of CD8⁺ T_{CM}, whereas CD8⁺ T cells cultured in the presence of IL-2 show characteristics of CD8⁺ T_{EFF} cells.^{38,39} It has been reported that T_{CM} or naive CD8⁺ T cells expressed little mRNA encoding BLT1 whereas BLT1 expression on T_{EFF} was upregulated.^{34,35}

LTB₄ CONTRIBUTES TO THE RECRUITMENT AND ACTIVATION OF NEUTROPHILS AND EOSINOPHILS

Studies in human have suggested the importance of LTB₄ in asthma. For example, it has been reported that the levels of 5-LO and LTA₄H were increased in the airways and circulating neutrophils of patients with asthma.^{40,41} Increased levels of LTB₄ have also been shown in the blood, BAL fluid, and exhaled breath condensates of patients with asthma.⁴²⁻⁴⁷ In contrast to the cysteinyl leukotrienes, which are potent mediators of bronchoconstriction,⁴⁸ LTB₄ is thought to be a proinflammatory mediator, and the classical major activities of LTB₄ are the recruitment, activation and prolongation of survival of myeloid leukocytes including neutrophils and eosinophils.^{13,49-52} As to a pathogenic role for neutrophils in asthma, large numbers of neutrophils have been found in the airways of patients with asthma who were suffering clinical exacerbations or status asthmaticus.⁵⁰⁻⁵³ Patients who suffered a sudden asthma-related death also showed increased numbers of neutrophils in the lungs.⁵⁴ A correlation between eosinophil numbers in the airways and disease severity⁵⁵ and a study utilizing mice lacking eosinophils⁵⁶ have indicated the pathological role of eosinophils in asthma. A study in mice indicated that LTB₄ and BLT1 participate in the early phase recruitment of eosinophils and neutrophils to the airways.⁵⁷ Therefore, LTB₄ might contribute to the pathogenesis of asthma through the recruitment and activation of neutrophils and eosinophils.

BLT1 CONTRIBUTES TO IL-13 PRODUCTION FROM T CELLS AND ALLERGIC AIRWAY RESPONSES

T cells are thought to play a key role in orchestrating the disease process through the production of a vari-

ety of cytokines in asthma. Several studies have shown that LTB4 may be important for cytokine secretion from T cells *in vitro*.⁵⁸⁻⁶¹ Recent studies have shown that LTB4 acts as an important attractant for differentiated T cells. *In vivo*, we have shown that the expression of BLT1 can play an important role in IL-13 production from T cells and the full development of allergen-induced AHR.⁶² Using BLT1 $-/-$ mice with a targeted disruption of the receptor, allergen-induced AHR was significantly reduced compared to BLT1 $+/+$ mice. Numbers of BAL eosinophils were similar in both strains of mice as were serum levels of antigen-specific IgE and IgG1. *In vivo* IL-13 production from lung cells was significantly reduced in the deficient mice following sensitization and challenge. BLT1 expression on CD4 $^{+}$ and CD8 $^{+}$ T cells may be critical in mediating effector function of lung T cells, especially a subset committed to IL-13 production because the numbers of IL-13 $+/CD4^{+}$ and IL-13 $+/CD8^{+}$ T cells in the lung were significantly lower than in BLT1 $+/+$ mice. Further, *in vitro* IL-13 production by lung BLT1 $-/-$ T cells was lower than in BLT1 $+/+$ T cells.⁶² Following transfer of antigen-primed BLT1 $+/+$ T, but not naive BLT1 $+/+$ T cells, the development of AHR and levels of IL-13 were fully restored in the BLT1 $-/-$ mice. These data suggest that the BLT1 contribution to the development of AHR may be linked to IL-13 production from recruited T cells. Following sensitization and challenge, the numbers of IL-13 $+/CD4$ and IL-13 $+/CD8$ in the spleen were not different between the 2 strains of mice, suggesting that BLT1 contributes more to the recruitment of IL-13 producing T cells to the lung rather than functional activation of these types of T cells. Turner *et al.* have also reported that CP-105696, an antagonist of the LTB4 receptor, suppressed AHR in a primate model,⁶³ consistent with our data. Using different strains of BLT1 mice, Terawaki *et al.* also reported attenuated AHR in BLT1-deficient mice, which were established independently from the ones we used. In their study, BLT1-deficient mice on a C57BL/6 background exhibited reduced AHR and decreased cytokine IL-13 in BAL.⁶⁴ The production of IL-4 and IL-5 as well as IL-13 from cultures of peribronchial lymphnode (PBLN) cells were also attenuated with BLT1 deficiency, suggesting that the LTB4-BLT1 pathway may be required for functional activation of Th2-type cells. Thus, the contribution of BLT1 to IL-13 production from lung T cells may be not only through recruitment of subsets of these cells capable of IL-13 production, but also the functional activation of these cells to produce IL-13.

Interestingly, airway responses induced by recombinant IL-13 may require an intact LTB4 pathway *in vivo*,⁶⁵ suggesting that the LTB4 pathway may also be involved in these IL-13-induced and dependent events. Since effector T cells in the lung are a source of IL-13 following allergen-challenge, this release of

IL-13 may further activate LTB4 production in the lung and serve to amplify or enhance the accumulation and activation of IL-13 producing effector T cells.

LTB4-DIRECTED ANTIGEN-SPECIFIC EFFECTOR CD8 $^{+}$ T-CELL TRAFFICKING IN ASTHMA

In contrast to the role of CD4 $^{+}$ T cells in allergic airway disease, the contribution of CD8 $^{+}$ T cells to the development of allergic airway disease is more controversial and has been more difficult to define. This may be due to the functional heterogeneity of CD8 $^{+}$ T cell subpopulations involved in different phases of the development of an allergic airway response. Currently, there is increasing evidence indicating the contribution of CD8 $^{+}$ T cells to allergic airway responses.⁴⁻¹⁰ We have recently shown that following sensitization and allergen exposure, mice deficient in the CD8 $^{+}$ chain developed less airway inflammation and AHR compared to wild-type mice.⁷ This was associated with decreased levels of the TH2 cytokine IL-13 in BAL fluid. Transfer of CD8 $^{+}$ T cells from sensitized, but not from naive donors, prior to allergen challenge fully reconstituted the development of airway inflammation, IL-13, and AHR in sensitized CD8-deficient mice, emphasizing that "allergen priming" is necessary for the supportive function of CD8 $^{+}$ T cells, similar to that shown with CD4 $^{+}$ T cells.⁶⁶ To further characterize the role of CD8 T cells, we differentiated antigen-specific T_{EFF} and T_{CM} from OVA₂₅₇₋₂₆₄ (SIIN-FEKL) peptide-specific TCR transgenic mice in the presence of IL-2 (T_{EFF}) and IL-15 (T_{CM}), respectively. Reconstitution of CD8 $-/-$ mice with T_{EFF} increased AHR, lung eosinophilia and IL-13 levels, whereas T_{CM} failed to do so. The T_{EFF} accumulated in the lung, whereas the T_{CM} were detected in the PBLN. Increased numbers of CD8 $^{+}/IL-13^{+}$ cells were found in the lungs of recipients following transfer of T_{EFF}. These data further link CD8 $^{+}$ T cells and effector CD8 $^{+}$ T cells in particular with the full development of AHR and airway inflammation.

As discussed above, the LTB4-BLT1 pathway appears to play an important role in the recruitment/activation of IL-13 producing T cells in the lung. As previously described, *in vitro* generated CD8 T_{EFF} express higher mRNA levels of the LTB4 high affinity receptor, BLT1, than CD8 T_{CM}.^{34,35} To investigate directly whether BLT1 expression was essential for the development of CD8-mediated allergen-induced AHR and inflammation, we adoptively transferred BLT1 $+/+$ or BLT1 $-/-$ CD8 or *in vitro* generated CD8 T_{EFF} into the CD8 $-/-$ mice.⁶⁷ Only CD8 or T_{EFF} expressing BLT1 were effective in fully reconstituting all of the responses, including IL-13, identifying the essential role of this LTB4 receptor on CD8 T cells. Further, only BLT1 $+/CD8^{+}/IL-13^{+}$ T cells were significantly increased in the lungs or BAL of sensitized and chal-

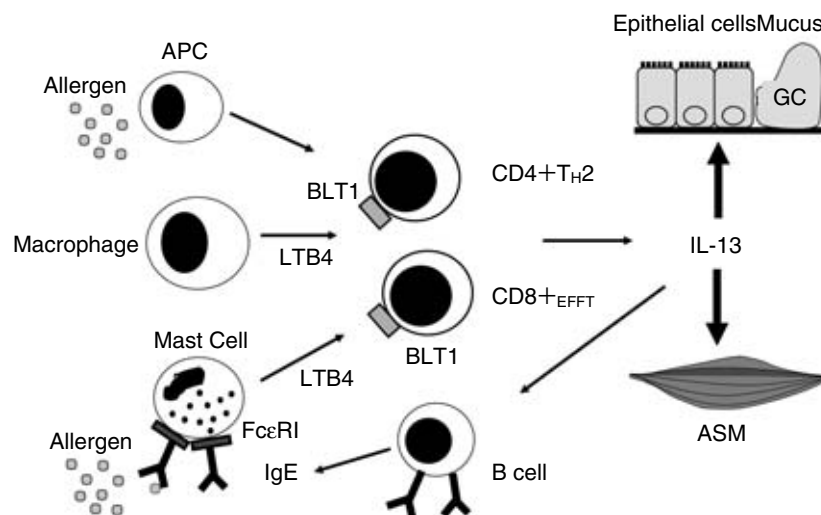


Fig. 1 Potential mechanisms of allergic airway disease in sensitized hosts following allergen exposure. Following allergen exposure, activation of mast cells via the high affinity Fc receptor for IgE (FcεRI) leads to mast cell activation, triggering release of mediators including lipid mediators such as leukotriene B4 (LTB4). LTB4-BLT1 (leukotriene B4 receptor 1) interaction leads to the recruitment/activation of allergen-specific CD4⁺ type-2 helper T cells (T_{H2}) and allergen-specific effector CD8⁺ T cells (T_{EFF}). In the mast cell-independent pathway, allergen exposure leads to recruitment of effector T cells likely through LTB4 generated by other cell types (e.g., macrophages). Effector T cells produce IL-13. IL-13 then acts on airway epithelial cells to induce goblet cell (GC) metaplasia and on airway smooth muscle (ASM) to induce increased responsiveness. APC, antigen presenting cells.

lenged recipients and in much higher numbers. Tager *et al.* reported that trafficking of T_{EFF} into the airway did not differ in the presence or absence of this receptor following adoptive transfer and airway challenge of naive (non-sensitized) recipients.³³ In contrast, using sensitized as opposed to naive recipient mice, we showed that migration of transferred BLT1^{-/-} T_{EFF} into the lung as well as BAL was significantly impaired compared to BLT1^{+/+} T_{EFF}. In this adoptive transfer model, the recipient mice were sensitized to OVA (plus alum) prior to OVA challenge. LTB4 production in the lungs of sensitized and challenged recipients should be significantly higher than challenged only recipients,⁶⁸ and these increased levels of LTB4 may play a pivotal role in enhancing the recruitment of transferred BLT1^{+/+} T_{EFF} into the lung. In a different lung disease model, BLT1 has also shown to contribute to the development of lung rejection and obliterative bronchiolitis by mediating effector CD8⁺ T cell trafficking into the lung.⁶⁹

Different members of the chemokine family are known to be subset-selective chemoattractants for T cells. CCL2 and CCL5 may be important in the recruitment of CD8⁺ T cells.³⁸ T_{EFF} were reported to migrate in response to CCL5 as well as LTB4 *in vitro*.³⁵ It appears that LTB4-dependent signals contribute at least one essential link to a chain of molecular events

that may be required for efficient recruitment of T_{EFF} to the allergic airways.

IGE-FCεRI-MAST CELL-CD8-BLT1-IL-13 CONNECTION

The studies in mice described above were generated in sensitized and challenged mice. This experimental model is believed to be IgE and mast cell-independent because mast-cell deficient mice and B-cell-deficient mice develop similar degrees of airway allergic responses as their respective normal littermates.^{70,71} Somewhat in parallel, we examined responses in a mast cell-dependent system. Briefly, using a 10-day allergen exposure approach, FcεRI-deficient mice failed to develop altered airway function, less airway inflammation, and lower IL-13 levels. Transfer of WT bone marrow-derived mast cells (BMMC) fully restored these responses in FcεRI^{-/-} mice and transferred mast cells could be detected in the tracheal preparations when derived from UBI-GFP mice, suggesting that IgE-FcεRI-mast cells are involved in the responses to 10 days of airway allergen exposure.⁷² IL-13 was shown to be critical to these responses as they were absent in IL-13^{-/-} mice and prevented in WT mice treated with an IL-13 receptor antagonist. Transfer of WT BMMC into IL-13^{-/-} mice failed to restore the responses, however,

transfer of IL-13-/- BMMC into FcεRI-/- mice did restore the responses, suggesting that IL-13 was indeed derived from another cell type other than the mast cell.⁷² CD8+ T cells were shown to be required for development of altered airway responsiveness.⁶

Using a passive sensitization model to avoid any potential interference on the host's ability to make IgE,⁷³ we examined a number of different mice. Here, mice were passively sensitized by injection of allergen-specific IgE prior to airway challenge. Mast cell-deficient, FcεRI-/-, IL-13-/-, BLT1-/-, and CD8-/- mice all failed to develop alterations in airway responsiveness following passive sensitization with OVA-specific IgE and limited airway allergen challenge (unpublished observations).⁷⁴ Recipient CD8-/- mice, reconstituted with CD8+ T_{EFF}, restored all of the responses. Moreover, only T_{EFF} expressing BLT1 but not BLT1-/- T_{EFF} were capable of doing so. The numbers of BLT1+ T_{EFF} cells were significantly higher than those of BLT1-/- T_{EFF} in the lungs of recipients. In addition, the induction of increased airway responsiveness and their accumulation in the lung following transfer of BLT1+ /CD8+ T_{EFF} could be blocked by administration of an LTB4 receptor antagonist. In parallel, we monitored BAL levels of LTB4. LTB4 levels in mast cell-deficient and FcεRI-deficient mice were significantly lower compared to their respective littermates following passive sensitization and challenge, suggesting that mast cells were the primary source of LTB4 following passive sensitization and allergen challenge. Thus, in both mast cell-independent and mast cell-dependent systems, there is strong support for an LTB4-BLT1-CD8-IL-13 module (Fig. 1).

CONCLUSIONS

Although the importance of LTB4 in allergic airway disease has been suggested for over two decades, recent experimental evidence now indicates a critical role for an LTB4-BLT1 pathway in IL-13 production from T lymphocytes and recruitment of antigen-specific effector CD8+ T cells in both mast cell dependent and independent allergic airway responses. The cumulative data indicate that control of this LTB4-BLT1 pathway should provide novel therapeutic opportunities for the treatment of asthma.

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REFERENCES

- Busse WW, Lemanske RF Jr. Asthma. *New Engl. J. Med.* 2001;**344**:350-362.
- Lee NA, Gelfand EW, Lee JJ. Pulmonary T cells and eosinophils: coconspirators or independent triggers of allergic respiratory pathology? *J. Allergy Clin. Immunol.* 2001;**107**:945-957.
- Oshiba A, Hamelmann E, Haczku A *et al.* Modulation of antigen-induced B and T cell responses by antigen-specific IgE antibodies. *J. Immunol.* 1997;**159**:4056-4063.
- Gonzalez MC, Diaz P, Galleguillos FR, Ancic P, Cromwell O, Kay AB. Allergen-induced recruitment of bronchoalveolar helper (OKT4) and suppressor (OKT8) T-cells in asthma. Relative increases in OKT8 cells in single early responders compared with those in late-phase responders. *Amer. Rev. Resp. Dis.* 1987;**136**:600-604.
- O'Sullivan S, Cormican L, Faul JL *et al.* Activated, cytotoxic CD8+ T lymphocytes contribute to the pathology of asthma death. *Amer. J. Resp. Crit. Care Med.* 2001;**164**:560-564.
- Hamelmann E, Oshiba A, Paluh J *et al.* Requirement for CD8+ T cells in the development of airway hyperresponsiveness in a murine model of airway sensitization. *J. Exp. Med.* 1996;**183**:1719-1729.
- Miyahara N, Takeda K, Kodama T *et al.* Contribution of antigen-primed CD8+ T cells to the development of airway hyperresponsiveness and inflammation is associated with IL-13. *J. Immunol.* 2004;**172**:2549-2558.
- Miyahara N, Swanson BJ, Takeda K *et al.* Effector CD8+ T cells mediate inflammation and airway hyperresponsiveness. *Nature Med.* 2004;**10**:865-869.
- Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5 and interferon-γ in airway CD4+ and CD8+ T cells in atopic asthma. *Amer. J. Resp. Crit. Care Med.* 2004;**171**:224-230.
- Isogai S, Taha R, Tamaoka M, Yoshizawa Y, Hamid Q, Martin JG. CD8+ alphaβ T cells can mediate late airway responses and airway eosinophilia in rats. *J. Allergy Clin. Immunol.* 2004;**114**:1345-1352.
- Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 1987;**237**:1171-1176.
- Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *New Engl. J. Med.* 1990;**323**:645-655.
- Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJ. Leukotriene B₄, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 1980;**286**:264-265.
- Serhan CN, Prescott SM. The scent of a phagocyte: Advances on leukotriene B₄ receptors. *J. Exp. Med.* 2000;**192**:F5-F8.
- Huang WW, Garcia-Zepeda EA, Sauty A, Oettgen HC, Rothenberg ME, Luster AD. Molecular and biological characterization of the murine leukotriene B₄ receptor expressed on eosinophils. *J. Exp. Med.* 1998;**188**:1063-1074.
- Tager AM, Luster AD. BLT1 and BLT2: the leukotriene B₄ (4) receptors. *Prostaglandins Leuko. Essent. Fatty Acids* 2003;**69**:123-134.
- Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol.* 1999;**17**:255-281.
- Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T. A G-protein-coupled receptor for leukotriene B₄ that mediates chemotaxis. *Nature* 1997;**387**:620-624.
- Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. A second leukotriene B₄ receptor, BLT2. A new therapeutic target in inflammation and immunological disorders.

- ders. *J. Exp. Med.* 2000;**192**:421-432.
20. Feinmark SJ, Cannon PJ. Endothelial cell leukotriene C4 synthesis results from intercellular transfer of leukotriene A4 synthesized by polymorphonuclear leukocytes. *J. Biol. Chem.* 1986;**261**:16466-16472.
 21. Macclouf JA, Murphy RC. Transcellular metabolism of neutrophil-derived leukotriene A4 by human platelets. A potential cellular source of leukotriene C4. *J. Biol. Chem.* 1988;**263**:174-181.
 22. Sala A, Rossoni G, Buccellati C, Berti F, Folco G, Macclouf J. Formation of sulphidopeptide-leukotrienes by cell-cell interaction causes coronary vasoconstriction in isolated, cell-perfused heart of rabbit. *Br. J. Pharmacol.* 1993;**110**:1206-1212.
 23. Bray MA, Ford-Hutchinson AW, Shipley ME, Smith MJ. Calcium ionophore A23187 induces release of chemokinetic and aggregating factors from polymorphonuclear leukocytes. *Br. J. Pharmacol.* 1980;**71**:507-512.
 24. Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 1983;**220**:568-575.
 25. Henderson WR, Jorg A, Klebanoff SJ. Eosinophil peroxidase-mediated inactivation of leukotrienes B4, C4 and D4. *J. Immunol.* 1982;**128**:2609-2613.
 26. Lee CW, Lewis RA, Tauber AI, Mehrotra M, Corey EJ, Austen KF. The myeloperoxidase-dependent metabolism of leukotrienes C4, D4 and E4 to 6-*trans*-leukotriene B4 diastereoisomers and the subclass-specific S-diastereoisomeric sulfoxides. *J. Biol. Chem.* 1983;**258**:15004-15010.
 27. Bosterling B, Trudell JR. Leukotriene B4 metabolism by hepatic cytochrome P-450. *Biochem. Biophys. Res. Commun.* 1983;**114**:850-854.
 28. Smith MJ, Ford-Hutchinson AW, Bray MA. Leukotriene B: a potential mediator of inflammation. *J. Pharm. Pharmacol.* 1980;**32**:517-518.
 29. Payan DG, Missirlian-Bastian A, Goetzl EJ. Human T-lymphocyte subset specificity of the regulatory effects of leukotriene B4. *Proc. Natl. Acad. Sci. U.S.A.* 1984;**81**:3501-3505.
 30. Leppert D, Hauser SL, Kishiyama JL, An S, Zeng L, Goetzl EJ. Stimulation of matrix metalloproteinase-dependent migration of T cells by eicosanoids. *FASEB J.* 1995;**9**:1473-1481.
 31. Bacon KB, Camp RD, Cunningham FM, Woollard PM. Contrasting *in vitro* lymphocyte chemotactic activity of the hydroxyl enantiomers of 12-hydroxy-5, 8, 10, 14-eicosatetraenoic acid. *Br. J. Pharmacol.* 1988;**95**:966-974.
 32. Huang WW, Garcia-Zepeda EA, Sauty A, Oettgen HC, Rothenberg ME, Luster AD. Molecular and biological characterization of the murine leukotriene B4 receptor expressed on eosinophils. *J. Exp. Med.* 1998;**188**:1063-1074.
 33. Tager AM, Bromley SK, Medoff BD *et al.* Leukotriene B4 receptor BLT1 mediates early effector T cell recruitment. *Nat. Immunol.* 2003;**4**:982-990.
 34. Goodarzi K, Goodarzi M, Tager AM, Luster AD, von Andrian UH. Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. *Nature Immunol.* 2003;**4**:965-973.
 35. Ott VL, Cambier JC, Kappler J, Marrack P, Swanson BJ. Mast cell-dependent migration of effector CD8⁺ T cells through production of leukotriene B4. *Nature Immunol.* 2003;**4**:974-981.
 36. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;**401**:708-712.
 37. Masopust D, Vezys V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 2001;**291**:2413-2417.
 38. Weninger W, Crowley MA, Manjunath N, von Andrian UH. Migratory properties of naive, effector, and memory CD8⁺ T cells. *J. Exp. Med.* 2001;**194**:953-966.
 39. Manjunath N, Shankar P, Wan J *et al.* Effector differentiation is not prerequisite for generation of memory cytotoxic T lymphocytes. *J. Clin. Invest.* 2001;**108**:871-878.
 40. Seymour ML, Rak S, Aberg D *et al.* Leukotriene and prostanoic pathway enzymes in bronchial biopsies of seasonal allergic asthmatics. *Am. J. Resp. Crit. Care Med.* 2001;**164**:2051-2056.
 41. Zaitzu M, Hamasaki Y, Matsuo M, Ichimaru T, Fujita I, Ishii E. Leukotriene synthesis is increased by transcriptional up-regulation of 5-lipoxygenase, leukotriene A4 hydrolase, and leukotriene C4 synthase in asthmatic children. *J. Asthma.* 2003;**40**:147-154.
 42. Shindo K, Fukumura M, Miyakawa K. Leukotriene B4 levels in the arterial blood of asthmatic patients and the effects of prednisolone. *Eur. Respir. J.* 1995;**8**:605-610.
 43. Sampson AP, Castling DP, Green CP, Price JF. Persistent increase in plasma and urinary leukotrienes after acute asthma. *Arch. Dis. Child* 1995;**73**:221-225.
 44. Wenzel SE, Trudeau JB, Kaminsky DA, Cohn J, Martin RJ, Westcott JY. Effect of 5-lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma. *Am. J. Respir. Crit. Care Med.* 1995;**152**:897-905.
 45. Zaitzu M, Hamasaki Y, Ishii K *et al.* Direct evidence that LTC4 and LTB4 but not TXA2 are involved in asthma attacks in children. *J. Asthma* 1998;**35**:445-448.
 46. Csoma Z, Kharitonov SA, Balint B, Bush A, Wilson NM, Barnes PJ. Increased leukotrienes in exhaled breath condensate in childhood asthma. *Am. J. Respir. Crit. Care Med.* 2002;**166**:1345-1349.
 47. Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. *J. Allergy Clin. Immunol.* 2002;**109**:615-620.
 48. Drazen JM. Comparative contractile responses to sulfidopeptide leukotrienes in normal and asthmatic human subjects. *Ann. N.Y. Acad. Sci.* 1988;**524**:289-297.
 49. Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Waltmann P, Kay AB. Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood* 1992;**79**:2952-2959.
 50. Rae SA, Smith MJH. The stimulation of lysosomal enzyme secretion from human polymorphonuclear leukocytes by leukotriene B4. *J. Pharm. Pharmacol.* 1981;**33**:616-617.
 51. Sumimoto H, Takeshige K, Minakami S. Superoxide production of human polymorphonuclear leukocytes stimulated by leukotriene B4. *Biochim. Biophys. Acta* 1984;**803**:271-277.
 52. Hebert MJ, Takano T, Holthofer H, Brady HR. Sequential morphologic events during apoptosis of human neutrophils. Modulation by lipoxygenase-derived eicosanoids. *J. Immunol.* 1996;**157**:3105-3115.
 53. Lamblin C, Gosset P, Tillie-Leblond I *et al.* Bronchial neutrophilia in patients with noninfectious status asthmaticus. *Am. J. Respir. Crit. Care Med.* 1998;**157**:394-402.
 54. Sur S, Crotty TB, Kephart GM *et al.* Sudden-onset fatal asthma. A distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? *Am. Rev. Respir. Dis.* 1993;**148**:713-719.

55. Bousquet J, Chanez P, Lacoste JY *et al.* Eosinophilic inflammation in asthma. *N. Engl. J. Med.* 1990;**323**:1033-1039.
56. Lee JJ, Dimina D, Macias MP *et al.* Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004;**305**:1773-1776.
57. Medoff BD, Tager AM, Jackobek R, Means TK, Wang L, Luster AD. Antibody-antigen interaction in the airway drives early granulocyte recruitment through BLT1. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2006;**290**:L170-178.
58. Farrar WL, Humes JL. The role of arachidonic acid metabolism in the activities of interleukin 1 and 2. *J. Immunol.* 1985;**135**:1153-1159.
59. Rola-Pleszczynski M, Chavaille PA, Lemaire I. Stimulation of interleukin 2 and interferon gamma production by leukotriene B4 in human lymphocyte cultures. *Prostaglandins. Leukot. Med.* 1986;**23**:207-210.
60. Arcoleo F, Milano S, D'Agostino P, Cillari E. Effect of exogenous leukotriene B4 (LTB4) on BALB/c mice splenocyte production of Th1 and Th2 lymphokines. *Intl. J. Immunopharmacol.* 1995;**17**:457-463.
61. Yamaoka KA, Kolb JP. Leukotriene B4 induces interleukin 5 generation from human T lymphocytes. *Eur. J. Immunol.* 1993;**23**:2392-2398.
62. Miyahara N, Takeda K, Miyahara S *et al.* Requirement for the leukotriene B4 receptor-1 in allergen-induced airway hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* 2005;**172**:161-167.
63. Turner CR, Breslow R, Conklyn MJ *et al.* *In vitro* and *in vivo* effects of leukotriene B4 antagonism in a primate model of asthma. *J. Clin. Invest.* 1996;**97**:381-387.
64. Terawaki K, Yokomizo T, Nagase T *et al.* Absence of leukotriene b4 receptor 1 confers resistance to airway hyperresponsiveness and th2-type immune responses. *J. Immunol.* 2005;**175**:4217-4225.
65. Vargaftig BB, Singer M. Leukotrienes mediate murine bronchopulmonary hyperreactivity, inflammation, and part of mucosal metaplasia and tissue injury induced by recombinant murine interleukin-13. *Amer. J. Resp. Cell Mol. Biol.* 2003;**28**:410-419.
66. Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K. Induction of airway mucus production by T helper 2 (Th2) cells: A critical role for interleukin 4 in cell recruitment but not mucus production. *J. Exp. Med.* 1997;**186**:1737-1747.
67. Miyahara N, Takeda K, Miyahara S *et al.* Leukotriene B4 receptor-1 is essential for allergen-mediated recruitment of CD8+ T cells and airway hyperresponsiveness. *J. Immunol.* 2005;**174**:4979-4984.
68. Henderson WR Jr, Lewis DB, Albert RK *et al.* The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J. Exp. Med.* 1996;**184**:1483-1494.
69. Medoff BD, Seung E, Wain JC *et al.* BLT1-mediated T cell trafficking is critical for rejection and obliterative bronchiolitis after lung transplantation. *J. Exp. Med.* 2005;**202**:97-110.
70. Takeda K, Hamelmann E, Joetham A *et al.* Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J. Exp. Med.* 1997;**186**:449-454.
71. Korsgren M, Erjefalt JS, Korsgren O, Sundler F, Persson CG. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. *J. Exp. Med.* 1997;**185**:885-892.
72. Taube C, Wei X, Swasey CH *et al.* Mast cells, FcεRI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J. Immunol.* 2004;**172**:6398-6406.
73. Oshiba A, Hamelmann E, Takeda K *et al.* Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. *J. Clin. Invest.* 1996;**97**:1398-1408.
74. Taube C, Miyahara N, Ott V *et al.* The leukotriene B4 receptor (BLT1) is required for effector CD8+ T cell-mediated, mast cell-dependent airway hyperresponsiveness. *J. Immunol.* 2006;**176**:3157-3164.