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Importance of the Leukotriene B₄-BLT₁ and LTB₄-BLT₂ Pathways in Asthma

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Abstract

For several decades, the leukotriene pathways have been implicated as playing a central role in the pathophysiology of asthma. The presence and elevation of numerous metabolites in the blood, sputum, and bronchoalveolar lavage fluid from asthmatics or experimental animals adds support to this notion. However, targeting of the leukotriene pathways has had, in general, limited success. The single exception in asthma therapy has been targeting of the cysteinyl leukotriene receptor 1, which clinically has proven effective but only in certain clinical situations. Interference with 5-lipoxygenase has had limited success, in part due to adverse drug effects. The importance of the LTB₄-BLT₁ pathway in asthma pathogenesis has extensive experimental support and findings, albeit limited, from clinical samples. The LTB₄-BLT₁ pathway was shown to be important as a neutrophil chemoattractant. Despite observations made more than two decades ago, the LTB₄-BLT₁ pathway has only recently been shown to exhibit important activities on subsets of T lymphocytes, both as a chemoattractant and on lymphocyte activation, as well as on dendritic cells, the major antigen presenting cell in the lung. The role of BLT₂ in asthma remains unclear. Targeting of components of the LTB₄-BLT₁ pathway offers innovative therapeutic opportunities especially in patients with asthma that remain uncontrolled despite intensive corticosteroid treatment.

Keywords

BLT₁; LTB₄; asthma; CD4 T lymphocytes; CD8 T lymphocytes

1.0 Introduction

Allergic asthma is a complex syndrome characterized by reversible airway obstruction, airway inflammation, and airway hyperresponsiveness (AHR). Allergen-specific memory T cells and IgE-specific antibodies play a central role in the development of these responses. In addition, many other cell types, including mast cells, eosinophils, basophils, neutrophils, and

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macrophages/dendritic cells (DCs) play roles under different conditions, often dictated by a host of cytokines and chemokines that direct their accumulation and activation in the lungs and airways. Initially viewed as an imbalance between CD4⁺ Th2 cytokine-producing cells (IL-4, IL-5, IL-13) and CD8⁺ Th1 (IFN- γ -producing) cells, it is now recognized that many T-cell subtypes play contributory roles and that both CD4⁺ and CD8⁺ T cells contribute to asthma pathophysiology and development of allergic airway responses [1].

1.1 Leukotriene B4 and asthma

As described elsewhere in this volume, leukotrienes are generated by the metabolism of arachadonic acid and are formed in different cell types as well as via transcellular metabolism involving multiple cells such as neutrophils, platelets, and vascular cells [2–4]. Human neutrophils and eosinophils synthesize LTB₄ and LTC₄, respectively [5, 6]. Monocytes and macrophages also synthesize both LTB₄ and the cysteinyl leukotrienes (cys-LTs) [7]. 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 [8, 9]. LTB₄ is also metabolized in the cells which produce this metabolite by a unique membrane bound cytochrome P450 enzyme [10].

LTB₄ at high concentrations binds and activates a nuclear receptor peroxisome proliferator-activated receptor α (PPAR α) [11]. The interaction with PPAR α induces the catabolic inactivation of LTB₄ and, in turn, limits LTB₄-related inflammation. Some LTB₄ receptor antagonists have also been reported to exhibit PPAR agonistic activity [12]. Thus, responses to LTB₄ could represent an integration of both pro-inflammatory and anti-inflammatory effects.

A number of studies have identified the importance of LTB₄ in asthma. Levels of 5-lipoxygenase (5-LO) and leukotriene A₄ hydrolase (LTA₄H) were increased in the airways and circulating neutrophils from patients with asthma [13, 14]. Increased levels of LTB₄ have been demonstrated in the blood, bronchoalveolar lavage (BAL) fluid, and exhaled breath condensates from asthmatics [15–20]. In contrast to the cys-LTs, which are potent mediators of bronchoconstriction [21], LTB₄ is a pro-inflammatory mediator with major activities on the recruitment, activation, and prolongation of survival of myeloid leukocytes, including neutrophils and eosinophils [22–25]. The contribution of neutrophils to asthma has been highlighted by the identification of a subtype of asthma as neutrophilic, as opposed to eosinophilic or pauci-cellular disease. Neutrophilic disease has been characterized in difficult to treat asthmatics who appear less steroid-responsive than those with a predominance of eosinophils [26]. Unlike eosinophils that are steroid-sensitive and undergo apoptosis in the presence of steroids, neutrophils are steroid-insensitive [1, 25]. Large numbers of neutrophils have been found in the airways of asthmatics suffering from an exacerbation of their disease or status asthmaticus [27]. In patients who suffered from a sudden asthma-related death, increased numbers of neutrophils were found in the lungs [28].

1.2 Receptors for LTB₄

LTB₄ mediates multiple biological functions through its interaction with two distinct receptors, LTB₄ receptor 1 (BLT1) and LTB₄ receptor 2 (BLT2). Although LTB₄ was one of the earliest leukocyte chemoattractants identified, characterization of the receptors was more difficult. The human high-affinity receptor, BLT1, was cloned by Yokomizo *et al.* in 1997 from differentiated HL-60 cells and provided a molecular tag for LTB₄ activities [29]. A second, low-affinity LTB₄ receptor, BLT2 was also identified by Yokomizo *et al.* [30] (see Chapter__). Both receptors are members of the G-protein-coupled seven-transmembrane-domain receptor (GPCR) superfamily, whose genes are located in close proximity to each other in 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. An important difference is their expression pattern and function in different cell types and it is the expression pattern of BLT1 and BLT2 that defines the activities of LTB₄: BLT1 is expressed primarily on leukocytes (granulocytes, macrophages, monocytes, DCs, and eosinophils), cells implicated in asthma pathogenesis. In contrast, BLT2 is expressed more ubiquitously [31]. The expression pattern is consistent with the notion that LTB₄ is a local inflammatory mediator, a chemoattractant for myeloid leukocytes [5, 32]. The potent leukocyte chemotactic activity of LTB₄ through BLT1 signaling has been well established [33]. In addition, functional BLT1 is now known to be expressed on nonmyeloid cells such as vascular smooth muscle cells, neural stem cells, and endothelial cells. In contrast to the specific activation of BLT1 by LTB₄, BLT2 is activated by several 12- and 15-lipoxygenase products in addition to LTB₄. Further, a cyclooxygenase metabolite 12-hydroxyheptadecatrienoic acid (12-HHT) binds to and activates BLT2 [4]. 12-HHT, which does not bind to BLT1, activates BLT2 at a 10-fold lower concentration than does LTB₄, suggesting that 12-HHT is a specific and high-affinity ligand for BLT2 [34]. In humans, BLT2 is widely distributed across multiple tissues. Mouse BLT2 is expressed predominantly in the small intestine, followed by skin, with low expression in the colon and spleen. BLT2 on mast cells mediates recruitment and accumulation of these cells in response to LTB₄ production at the sites of inflammation.

Although the LTB₄-BLT1 axis and GPCR signaling is known to promote inflammation, no studies have defined the binding proteins that modulate LTB₄-BLT1 signaling. Receptor for advanced glycation end products (RAGE) is a type I transmembrane receptor that belongs to the immunoglobulin superfamily [35] and plays a role in several inflammatory diseases. LTB₄-dependent ERK phosphorylation in neutrophils and LTB₄-dependent neutrophil accumulation in a murine peritonitis model were significantly attenuated in RAGE-deficient mice [36]. RAGE interacts with BLT1 and modulates LTB₄-BLT1 signaling through potentiation of the MEK-ERK pathway [36].

2.0 BLT1 expression on lymphocytes

Limited BLT1 expression was initially shown on naive lymphocytes [31, 37–40]. However, recent studies have suggested that it also acts as an important attractant for differentiated T cells.

2.1 BLT1 expression on T cells

BLT1 expression on mouse CD4⁺ T cells that have been differentiated *in vitro* to effector phenotypes has been reported [31]. CD4⁺ T cells that were activated *in vitro* under non-polarizing (Th0), Th1-polarizing, or Th2-polarizing conditions demonstrated increased levels of mRNA encoding BLT1 compared to naive cells which expressed little BLT1 [41]. 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 [41].

BLT1 expression on mouse CD8⁺ T cells differentiated *in vitro* in the presence of IL-2 or IL-15 to effector phenotypes has also been reported [42, 43]. Antigen-experienced populations 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 ascribed to these cells [44–46]. 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 acquired the phenotypic and functional characteristics of CD8⁺ T_{CM}, whereas CD8⁺ T cells cultured in the presence of IL-2 showed characteristics of CD8⁺ T_{EFF} cells [46, 47]. T_{CM} or naive CD8⁺ T cells expressed little mRNA encoding BLT1 whereas BLT1 expression on T_{EFF} was markedly upregulated [42, 43].

2.2 BLT1 expression on T cells contributes to IL-13 production and allergic airway responses

In asthma, T cells are thought to play a key role in orchestrating the disease process through the production of a variety of cytokines and other mediators. Several studies have shown that LTB4 may be important for enhancing cytokine secretion from T cells *in vitro* [48–51]. Other studies have shown that LTB4 acts as an important attractant for differentiated T cells. *In vivo*, we demonstrated that the expression of BLT1 played an important role in IL-13 production from T cells and the full development of lung allergic responses and allergen-induced AHR [52]. Using BLT1^{−/−} mice with a targeted disruption of the receptor, allergen-induced AHR was significantly reduced compared to (wild-type, WT) 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⁺ (Th2) and IL-13⁺/CD8⁺ Tc2 cells in the lung were significantly lower in BLT1^{−/−} mice compared to BLT1^{+/+} mice. Further, *in vitro* IL-13 production by lung BLT1^{−/−} T cells was lower than in BLT1^{+/+} T cells [52]. Following transfer of antigen-primed BLT1^{+/+} but not naive BLT1^{+/+} T cells, the development of AHR and levels of IL-13 were fully restored in the BLT1^{−/−} mice. These data suggested that the BLT1 contribution to the development of AHR was 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, indicating that BLT1 contributed 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 reported that CP-105696, an antagonist of the LTB4 receptor, suppressed AHR in a primate model [53], consistent with these data. Using a second strain of BLT1-deficient mice established independently, Terawaki *et al.* also reported attenuated AHR in BLT1-deficient mice. In their study, BLT1-deficient mice on a C57BL/6 background exhibited reduced AHR and decreased IL-13 levels in BAL fluid [54]. The production of IL-4 and IL-5 as well as IL-13 from cultures of peribronchial lymph node (PBLN) cells was also attenuated in BLT1-deficient mice, suggesting that the LTB4-BLT1 pathway may be required for functional activation of Th2/Tc2-type cells. Thus, the contribution of BLT1 to IL-13 production from lung T cells may not only be through recruitment of subsets of cells capable of IL-13 production, but also the functional activation of these cells to produce IL-13.

The airway responses induced by recombinant IL-13 may, in turn, require an intact LTB4 pathway *in vivo* [55], suggesting that the LTB4 pathway may also be involved in both IL-13-induced and IL-13-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 amplify or enhance the accumulation and activation of IL-13 producing effector T cells.

2.3 LTB4-directed antigen-specific effector CD8+ T-cell trafficking in asthma

In contrast to the role of CD4+ Th2 cells in allergic airway disease, the contribution of CD8+ Tc2 cells to the development of allergic airway disease is more controversial and has been more difficult to define. Although not dissimilar to the well-described heterogeneity of CD4+ T cells, the functional heterogeneity of CD8+ T cell subpopulations involved in different phases of the development of an allergic airway response has been less well defined. There is increasing evidence pointing to the contribution of CD8+ T cells to allergic airway responses in asthmatics and mice [56–63]. Following sensitization and allergen exposure, mice deficient in the CD8 α chain developed less airway inflammation and AHR compared to WT mice [59] and this was associated with decreased levels of the type 2 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+ Tc2 cells, similar to that shown with CD4+ Th2 cells [64]. To further characterize the role of CD8+ T cells, antigen-specific T_{EFF} and T_{CM} from OVA_{257–264} (SIINFEKL) peptide-specific TCR transgenic mice were differentiated in the presence of IL-2 (T_{EFF}) or IL-15 (T_{CM}). Reconstitution of CD8 $^{-/-}$ mice with T_{EFF} increased AHR, lung eosinophilia, and IL-13 levels, whereas reconstitution with T_{CM} failed to do so. T_{EFF} accumulated in the lung, whereas T_{CM} were only detected in the PBLN. Increased numbers of CD8+/IL-13+ cells were found in the lungs of recipients following transfer of T_{EFF} [60, 65]. These data further established the role of CD8+ T cells and effector CD8+ Tc2 cells in particular, with the full development of allergen-induced AHR and airway inflammation.

Taken together, these data indicated that the LTB₄-BLT1 pathway played an important role in the recruitment/activation of IL-13-producing T cells in the lung (Figure 1). CD8 T_{EFF} generated *in vitro* expressed higher BLT1 mRNA levels than CD8 T_{CM} [42, 43]. To investigate whether BLT1 expression was essential for the development of CD8+ T cell-mediated allergen-induced AHR and inflammation, we adoptively transferred BLT1^{+/+} or BLT1^{-/-} CD8+ T cells or *in vitro* generated CD8 T_{EFF} into CD8^{-/-} mice [65]. Only CD8+ T cells or CD8 T_{EFF} expressing BLT1 were effective in fully reconstituting all of the responses, including IL-13, supporting the importance of expression of the high affinity 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 challenged recipients. Initial reports suggested that trafficking of T_{EFF} into the airways did not differ in the presence or absence of this receptor following adoptive transfer and airway challenge of naive (non-sensitized) recipients [41]. 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 in the BAL fluid 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 was significantly higher than in challenged only recipients, and these increased levels of LTB₄ likely played a pivotal role in enhancing the recruitment of transferred BLT1^{+/+} T_{EFF} into the lung [66]. In a different lung disease model, BLT1 has also been shown to contribute to the development of lung rejection and obliterative bronchiolitis by mediating effector CD8+ T cell trafficking into the lung [67].

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 [46]. T_{EFF} were reported to migrate in response to CCL5 as well as LTB₄ *in vitro* [43]. It appears that LTB₄-dependent signals contribute at least one essential link to a chain of molecular events that may be required for efficient and effective recruitment of T_{EFF} to the allergic airways. Together, these data identify an essential role for BLT1 expression on CD8+ T cells for their recruitment to the lung.

Support for the LTB₄-BLT1 axis in CD8+ T cell trafficking was generated in a model of contact dermatitis, oxazolone-induced skin lesions characterized by infiltration of neutrophils and CD8+ T lymphocytes [68]. BLT1 deficiency or blockade of LTB₄ or BLT1 by antagonists resulted in significantly reduced neutrophil and skin-infiltrating CD8+ T lymphocyte accumulation. Further, neutrophil depletion resulted in markedly reduced LTB₄ synthesis and CD8+ T cell infiltration. Subcutaneous injection of LTB₄ in neutrophil-depleted mice restored the infiltration of CD8+ T cells into the skin. Thus, in ways perhaps similar to asthma models, the LTB₄-BLT1 axis contributed to the contact dermatitis by mediating skin recruitment of neutrophils, a major source of LTB₄, that directed CD8+ T lymphocyte homing to antigen-challenged skin.

2.4 IgE-FcεRI-mast cell-CD8-BLT1-IL-13 connection

The data 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 developed similar degrees of airway allergic responses as their respective normal littermates [69, 70]. In parallel, we examined responses in a mast cell-dependent system which involved a 10-day airway allergen exposure approach in the absence of systemic sensitization [71]. In this model, 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 labeled and transferred mast cells could be detected in the tracheal preparations, suggesting that IgE-FcεRI⁺ mast cells were involved in the responses to 10 days of airway allergen exposure. IL-13 was shown to be critical for 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 restored the responses, suggesting that IL-13 was indeed derived from another cell type other than the mast cell [71]. This other cell type required for development of altered airway responsiveness was shown to be a CD8⁺ T cell [52, 58, 59] (Figure 1).

Using a passive sensitization model to avoid potential interference with the ability of the recipient to make IgE, we examined the responses in different strains of mice. Mice were passively sensitized by injection of allergen (OVA)-specific IgE prior to airway challenge [72]. 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 airway allergen challenge [71]. Recipient CD8^{-/-} mice, reconstituted with CD8⁺ T_{EFF}, restored all of the lung allergic responses. Moreover, only CD8⁺ 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 numbers 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- and FcεRI-deficient mice were significantly lower compared to their respective littermates following passive sensitization and challenge, suggesting that mast cells were an important source of LTB4 following passive sensitization and allergen challenge. Thus, in both mast cell-independent and mast cell-dependent systems, there was strong support for an LTB4-BLT1-CD8-IL-13 pathway in the development of experimental asthma. An important corollary to this involvement of CD8⁺ T cells in asthma pathogenesis was the demonstration that these CD8⁺ T_{EFF} cells, critical to experimental asthma development, were steroid-insensitive compared to CD4⁺ T cells and CD8⁺ T_{CM} [73].

3.0 DC expression of BLT1

3.1 BLT1 on DCs in development of type 2 cytokine responses and AHR

A critical step in the activation of T cells is the uptake, processing, and presentation of antigen by antigen presenting cells (APCs). Among the different types of APCs, lung DCs play a key role in the initiation of immune responses in the airways. DCs recognize and take up antigen in the peripheral tissues and process them. After antigen uptake and processing,

DCs mature and migrate to secondary lymphoid organs via the activation of chemotactic receptors [74, 75]. DCs then present processed peptides to the surface-bound MHC molecules which are recognized by T cells initiating T cell priming [76, 77]. Extracellular stimuli such as chemokines and some lipid mediators such as prostaglandins and cys-LTs stimulate DC chemotaxis [76–78]. DCs also express BLT1. To determine the role of BLT1 on DCs, bone marrow-derived DCs (BMDCs) were generated from BLT1^{−/−} and BLT1^{+/+} (WT) mice [79]. Naive BALB/c mice received OVA-pulsed BLT1-deficient (BLT1^{−/−}) BMDCs or WT BMDCs intratracheally and were then challenged with OVA on 3 consecutive days. Compared to recipients of WT BMDCs, mice that received BLT1^{−/−} BMDCs developed significantly lower AHR to inhaled methacholine, lower goblet cell metaplasia, reduced eosinophilic infiltration in the airways, and decreased levels of type 2 cytokines in the BAL fluid. Migration of BLT1^{−/−} BMDCs into PBLN was significantly impaired compared to transfer of BLT1^{+/+} BMDCs. These data suggested that BLT1 expression on DCs was required for migration of DCs to regional lymph nodes as well as for the development of AHR and airway inflammation (Figure 2).

3.2 Essential role of LTA4 hydrolase in LTB4 generation and the DC response

To further confirm that the migration of DCs into PBLN was dependent on the LTB4-BLT1 axis, the accumulation of DCs in the PBLN of LTA4H^{+/+} and LTA4H^{−/−} mice, which failed to produce LTB4 *in vivo* were compared [79]. DCs from the bone marrow of LTA4H^{+/+} mice were generated and then pulsed with OVA, and these BMDCs were transferred into LTA4H^{+/+} or LTA4H^{−/−} mice intratracheally. The numbers of OVA-pulsed BMDCs recovered 24 and 48 hrs later from PBLN of LTA4H^{−/−} mice were significantly lower than from PBLN of LTA4H^{+/+} mice. The reduced DC accumulation in the regional lymph nodes of the LTB4-deficient mice further supports the notion that recruitment of DCs to the regional lymph nodes in the airways was dependent on the LTB4-BLT1 pathway (Figure 2).

Taken together, the preclinical data suggest that BLT1 expression on a variety of cells, including T lymphocytes subsets and DCs, all implicated in asthma pathogenesis, plays important roles in their recruitment and activation in the lungs, BAL fluid, and regional lymph nodes. Despite some initial failures of pharmacologic targeting of these pathways in preclinical studies, the data identify the importance of the LTB4-BLT1 pathway in asthma pathogenesis and as a valid target for controlling allergen-induced AHR and eosinophilic airway disease with the potential to improve the treatment of asthma.

4.0 Human asthma

4.1 BLT1⁺-CD8⁺-IL-13⁺ T cells in the lungs and airways of asthmatics

We have shown that a subset of CD8⁺ T lymphocytes expressing BLT1 and producing IL-13 was present in significantly increased numbers in the airways of patients with asthma compared to healthy subjects [80, 81]. The frequency of these cells was inversely related to airflow obstruction determined by measurements of FEV₁ and FEF_[25–75] percent predicted values, suggesting a pathogenic role in asthma airway obstruction. These data identified a subset of CD8⁺ T lymphocytes expressing BLT1 and producing IL-13, similar to effector

memory CD8⁺ T cells in the experimental mouse models, that populated the airways of asthmatic subjects.

Several studies have reported increased CD8⁺ T lymphocyte numbers in asthmatic airways [82–85] or after segmental allergen challenge [86]. However, the role of these cells in the pathogenesis of asthma has remained controversial. Early studies speculated that CD8⁺ T lymphocytes might have a protective role in asthma [56, 83]. In other studies, asthmatics with higher percentages of BAL CD8⁺ T lymphocytes had lower FEV₁ and PC₂₀ values compared to asthmatics with lower percentages of these cells [87], suggesting that the relative abundance of these cells in asthmatic airways might be associated with worse lung function outcomes. The number of CD8⁺ T lymphocytes in the airways predicted the annual decline of FEV₁ in asthmatics [88] and transcriptome analyses showed that severe asthma was indeed associated with activation of circulating CD8⁺ and not CD4⁺ T cells [89].

4.2 Peripheral blood CD4⁺ and CD8⁺ T cells differentially express BLT1

To extend these observations, we compared the phenotypes and activation outcomes of peripheral blood CD4⁺ and CD8⁺ T cells in subsets of asthmatics defined as steroid-sensitive (SS) or steroid-resistant (SR) [90]. The percentages of cells which expressed BLT1 were higher in asthmatics than in non-asthmatics and higher in SR asthmatics. The highest percentages of BLT1-expressing cells were detected among activated CD8⁺ T cells. This increase in BLT1-expressing cells in SR asthmatic CD8⁺ T cells was maintained in the presence of corticosteroid. Thus, as in the mouse [73], the CD8⁺ T cells were insensitive to corticosteroids, unlike the CD4⁺ T cells. These data identified significant differences between CD4⁺ and CD8⁺ T cells and between SS and SR asthmatics, especially in terms of numbers of BLT1⁺ CD8⁺ T cells and their relative corticosteroid insensitivity and capacity for IL-13 production. The upregulated BLT1 receptor was shown to be functional as determined by intracellular Ca²⁺ mobilization in response to ligation of the BLT1 receptor by LTB₄. CD8⁺ T cells produced the highest levels of the cytokine IL-13. SR asthmatic CD4⁺ T cells produced the lowest levels of the anti-inflammatory cytokine IL-10 and IFN- γ levels were the lowest in cultures of SR asthmatic CD4⁺ and CD8⁺ T cells. Importantly, CD8⁺ T cell expansion, especially in SR asthmatics, was unaffected by inclusion of a corticosteroid in the cultures compared to CD4⁺ T cells, supporting their role in steroid-refractory asthma [1].

4.3 Asthma severity is correlated with LTB₄ levels and numbers of CD8⁺ T cells

Although LTB₄ was initially described as a major chemoattractant for myeloid leukocytes [5], LTB₄ was shown several decades ago to bind to a small proportion of human peripheral blood T cells [37]. Consistent with these findings, LTB₄ was shown to induce chemotaxis of human peripheral blood lymphocytes [40]. Elevated levels of LTB₄ have been reported in various allergic diseases and these levels have been related to disease activity and response to treatment. LTB₄ levels were increased in the sputum, plasma, and BAL fluid of asthmatics patients but not in healthy subjects [16, 91]. Many studies have shown that LTB₄, although perhaps not a primary mediator of allergic disease, may be important in specific conditions such as severe persistent asthma [16]. Both BAL LTB₄ levels [92] and bronchial wall CD8⁺ T cell numbers have correlated with disease severity [88]. Despite the somewhat

limited characterization of the LTB₄-BLT1-CD8-IL-13 pathway in asthmatics, together with the data from experimental models of asthma in mice there is support for the importance of this steroid-insensitive pathway in steroid-resistant (refractory) asthma [1].

5.0 BLT2

5.1 BLT2 in lung disease

Although BLT2 has been considered as a pro-inflammatory receptor similar to BLT1, others have demonstrated that BLT2-deficient mice exhibit, in an experimental model of asthma, enhanced airway eosinophilia and increased IL-13 levels in the lungs without influencing IL-4 and IgE production [93]. IL-13-producing CD4⁺ T cells were increased in the lungs of BLT2-deficient mice after allergen challenge. Silencing of BLT2 by siRNA enhanced IL-13 production in these CD4⁺ T cells. Expression of BLT2 mRNA in CD4⁺ T cells from asthmatics was shown to be reduced compared to control subjects. These data suggested that BLT2 may in fact protect the lungs from allergic airway inflammation, whereas impaired BLT2 expression on CD4⁺ T cells may contribute to the development of asthma. Thus, in contrast to BLT1, BLT2 on CD4⁺ T cells may function as an anti-inflammatory receptor in asthma. This potential protective and anti-inflammatory role of BLT2 was supported in a study showing that dextran sodium sulfate-induced colitis was exacerbated in BLT2-deficient mice [94]. However, in contrast to its putative protective effects, the BLT2 antagonist LY255283 or anti-sense BLT2 suppressed allergen-induced airway inflammation in a mouse model [95]. Since LY255283 can be a potent antagonist for both BLT1 as well as BLT2 [96], the inhibition of BLT1 by LY255283 may have led to the anti-inflammatory effects seen in the experiments.

Immunohistochemical analyses of bronchial biopsy specimens from asthmatics revealed increased expression of BLT2 in airway epithelial layers and microvascular endothelium [95]. Elevated expression of BLT2 mRNA in airway fibroblasts was also found in asthmatic subjects [97]. A recent study demonstrated that cigarette smoke extracts increased neutrophil adhesion to bronchial epithelial cells via BLT2 *in vitro* [98]. Thus, unlike the extensive characterization of the role of BLT1 in airway disease, the precise role of BLT2 in airway structural cells in asthma remains unclear.

Summary and Conclusions

Recent findings, although incomplete, suggest different functions of BLT1 and BLT2 in the pathogenesis of asthma. Despite the failure in initial clinical trials to demonstrate therapeutic benefits of LTB₄ receptor antagonists in asthma, more selective BLT1 antagonists may hold greater promise and prove to be more effective. Targeting BLT2 may also represent a novel therapeutic option for certain lung diseases. The complexity of asthma and the extent of gene-environment interactions that dictate the activation of different pathways at different stages of the disease poses many therapeutic challenges. Extrapolating findings in experimental models of disease to human conditions is fraught with many problems. Nonetheless, the evidence for the importance of the LTB₄-BLT1 pathway in experimental asthma pathogenesis and the limited, but supportive clinical data in asthmatics, underscore the need for better pharmacological targeting of components of this pathway.

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Figure 1.

LTB₄-BLT1-CD8⁺ T cell-IL-13 pathway in asthma pathogenesis. Following allergen ligation of IgE on the high affinity IgE receptor (FcεRI), mast cells are activated resulting in the production and release of LTB₄. Activated CD8⁺ T_{EFF} increase expression of the high affinity receptor BLT1, ligation of the receptor by LTB₄ results in the migration of CD8⁺ T_{EFF} to the lung where in the presence of IL-4, the cells transdifferentiate through a cascade of molecular events resulting in IL-13 release [1].

Figure 2.

Importance of the LTB₄-BLT1 pathway in dendritic cell activation and migration to regional lymph nodes resulting in CD8+ T_{EFF} cell activation. 1) In the lung mast cells, macrophages or neutrophils are activated releasing LTB₄. 2) Lung DCs expressing BLT1 and exposed to antigen (Ag), increase BLT1 expression as well as CCR7, resulting in their migration to the regional lymph nodes (PBLN). 3) Here, the antigen-presenting DCs encounter naive CD8+ T cells resulting in activation, priming, and increased BLT1 expression. 4) Activated CD8+ T_{EFF} migrate to the airways following ligation of BLT1 by LTB₄. 5) In the atopic environment in the lungs of allergic patients, IL-4 results in the transdifferentiation of CD8+ T_{EFF} for IL-13 production, resulting in AHR, mucus production, and airway inflammation. Ag: antigen TCR; TCR: T cell receptor; MHC: major histocompatibility complex.