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, Tcells

INTRODUCTION

Allergic asthma is a complex syndrome which has been characterized by airway obstruction, airway inflammation and airway hyperresponsiveness (AHR). Allergen-specific memory T cells and antibody are thought to play a central role in the development of these responses. 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.

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. LTB4 is a chemoattractant for leukocytes, including neutrophils, macrophages, monocytes, and eosinophils. LTB4 has classically been thought to be an important mediator of the early phase of the asthmatic response to inhaled allergens. Recently, the receptors for LTB4 have been identified, and LTB4 is thought to activate leukocytes through a G protein-coupled cell surface receptor, BLT1. As we discuss 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). 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.
The leukotrienes are formed in different cell types as well as via transcellular metabolism involving multiple cells such as neutrophils, platelets, and vascular cells. Human neutrophils and eosinophils synthesize LTB4 and LTC4, respectively. Monocytes and macrophages also synthesize both LTB4 and the cysteinyl LTs. LTC4 is metabolized to LTD4 and LTE4 by the cells in which this mediator is formed. In addition, the cysteinyl LTs can be transformed into 6-trans-LTB4 by hypochlorous acid, which is generated during the respiratory burst in leukocytes. LTB4 is also metabolized in the cells which produce this metabolite, by a unique membrane bound cytochrome P450 enzyme.

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. A second, low-affinity LTB4 receptor, BLT2 was also identified by Yokomizo et al. 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.

LTB4 was classically described as a chemoattractant for myeloid leukocytes, and BLT1 was shown to be expressed on granulocytes, monocytes, and eosinophils, and to a lesser extent on naive lymphocytes. These findings were consistent with the classical notion that LTB4 is a local inflammatory mediator. However, recent studies have suggested that other receptors may be important for differentiated T cells. Subsequently, BLT1 expression on mouse CD4+ T cells has been reported. CD4+ T cells that were activated in vitro to effector phenotypes have 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 naïve cells, which expressed little BLT1. By contrast, expression of BLT2 by naïve 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.

BLT1 expression on mouse CD8+ T cells that have been differentiated in vitro to effector phenotypes has also been reported. 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 recently ascribed to these cells. Antigen-experienced central memory CD8+ T cells (Tcm) are CD62Lhi/CCR7hi and home preferentially to lymph nodes. Effector CD8+ T cells (TEFF) are CD62Llo/CCR7lo 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+ Tcm, whereas CD8+ T cells cultured in the presence of IL-2 show characteristics of CD8+ TEFF. It has been reported that Tcm or naïve CD8+ T cells expressed little mRNA encoding BLT1 whereas BLT1 expression on TEFF was upregulated.

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. Increased levels of LTB4 have also been shown in the blood, BAL fluid, and exhaled breath condensates of patients with asthma. In contrast to the cysteinyl leukotrienes, which are potent mediators of bronchoconstriction, 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.

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. Patients who suffered a sudden asthma-related death also showed increased numbers of neutrophils in the lungs. A correlation between eosinophil numbers in the airways and disease severity and a study utilizing mice lacking eosinophils 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. 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. 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. 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. Following transfer of antigen-primed BLT1+/+ T, but not naive BLT1+/+ T cells, the development of AHR and levels of IL-13 are 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, 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. The production of IL-4 and IL-5 as well as IL-13 from cultures of peribronchial lymph node (PBLN) cells were also attenuated with BLT1 deficiency, suggesting that the LT4-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 LT4 pathway in vivo, suggesting that the LT4 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 LT4 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. 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. 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. To further characterize the role of CD8 T cells, we differentiated antigen-specific TEFF and TCM from OVA257-264 (SIINFEKL) peptide-specific TCR transgenic mice in the presence of IL-2 (TEFF) and IL-15 (TCM), respectively. Reconstitution of CD8−/− mice with TEFF increased AHR, lung eosinophilia and IL-13 levels, whereas TCM failed to do so. The TEFF accumulated in the lung, whereas the TCM were detected in the PBLN. Increased numbers of CD8+/IL-13+ cells were found in the lungs of recipients following transfer of TEFF. 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 LT4-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 cells express higher mRNA levels of the LT4 high affinity receptor, BLT1, than CD8 TCM. 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 TFEFF into the CD8−/− mice. Only CD8 or TFEFF expressing BLT1 were effective in fully reconstituting all of the responses, including IL-13, identifying the essential role of this LT4 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 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. 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, 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.

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. T_{EFF} were reported to migrate in response to CCL5 as well as LTB4 in vitro. 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. 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. 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. CD8+ T cells were shown to be required for development of altered airway responsiveness.

Using a passive sensitization model to avoid any potential interference on the host's ability to make IgE, 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). Recipient CD8−/− mice, reconstituted with CD8+ T Eff, restored all of the responses. Moreover, only T eff expressing BLT1−/− were capable of doing so. The numbers of BLT1+ T Eff cells were significantly higher than those of BLT−/− 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 LT B4 receptor antagonist. In parallel, we monitored BAL levels of LT B4. LT B4 levels in mast cell-deficient and FcεRI-deficient mice were significantly lower compared to their respective littermates following passive sensitization and allergen challenge. Thus, in both mast cell-independent and mast cell-dependent systems, there is strong support for an LT B4-BLT1-CD8-IL-13 module (Fig. 1).

CONCLUSIONS

Although the importance of LT B4 in allergic airway disease has been suggested for over two decades, recent experimental evidence now indicates a critical role for an LT B4-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 LT B4-BLT1 pathway should provide novel therapeutic opportunities for the treatment of asthma.

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