The Role of Leukotriene B₄ in Allergic Diseases

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ABSTRACT
Leukotriene B₄ (LTB₄) is a lipid mediator with potent chemoattractant properties and that is rapidly generated from activated innate immune cells such as neutrophils, macrophages, and mast cells. Elevated levels of LTB₄ have been reported in various allergic diseases and these levels have been related to disease activity and response to treatment. Recent studies using LTB₄ receptor-1 (BLT1) antagonists or BLT1-deficient mice have revealed that ligation of BLT1 by LTB₄ is important for the activation and recruitment of inflammatory cells including neutrophils, eosinophils, monocytes/macrophages, mast cells, dendritic cells, and more recently, effector T cells to inflamed tissues in various inflammatory diseases. The LTB₄/BLT1 pathway appears to play an important role in the pathogenesis of severe persistent asthma, aspirin- and exercise-induced asthma, allergic rhinitis, and atopic dermatitis together with other mediators including cysteinyl leukotrienes, cytokines, and chemokines. LTB₄ production is in general resistant to corticosteroid treatment. In fact, corticosteroids can upregulate BLT1 expression on corticosteroid-resistant inflammatory cells such as neutrophils, monocytes, and effector memory CD8⁺ T cells. As a result, this corticosteroid-resistant LTB₄/BLT1 pathway may contribute to the development of inflammation in allergic diseases that do not respond to the introduction of corticosteroids. Inhibition of this pathway has potential therapeutic benefit in various allergic diseases that have involvement of corticosteroid-insensitivity.

KEY WORDS
allergic conjunctivitis, allergic rhinitis, asthma, atopic dermatitis

INTRODUCTION
The leukotrienes (LTs), a family of proinflammatory lipid mediators, play an important role in the pathogenesis of allergic inflammation and are divided into two classes: the chemoattractant LTB₄ and the spasmodenic cysteinyl LTs [CysLTs: LTC₄, LTD₄, and LTE₄] also known classically as slow-reacting substance of anaphylaxis (SRS-A).¹,³ These molecules are generated in leukocytes from membrane arachidonic acid through the action of 5-lipoxygenase (5-LO). LTB₄ is rapidly generated from membrane phospholipids of activated innate immune cells by the sequential enzymatic actions of cytosolic phospholipase A₂ (cPLA₂), 5-LO, and LTA₄ hydrolase.¹,³ LTB₄ serves as a potent chemoattractant through ligation of the high affinity LTB₄ receptor-1 (BLT1) on target cells.¹,² Various inflammatory diseases, including asthma,⁴,⁹ allergic rhinitis,¹⁰-¹³ atopic dermatitis,¹⁴-¹⁶ allergic conjunctivitis,¹⁷,¹⁸ rheumatoid arthritis,¹⁹ chronic obstructive pulmonary disease (COPD),²⁰,²¹ obliterative bronchiolitis after lung transplantation,²² and interstitial lung diseases²³,²⁴ are associated with increased levels of LTB₄ and/or BLT1 expression and in some of these diseases LTB₄ levels reflect disease activity and are decreased by treatment.⁷,¹²,¹³,¹⁸ Many studies have shown that LTB₄ is not a primary mediator of allergic diseases, but may be important in specific conditions such as severe persistent asthma,²⁵,²⁶ exercise- or aspirin-induced asthma,⁷,²⁷,²⁸ allergic rhinitis,¹⁰,¹¹ and atopic dermatitis.¹⁴-¹⁶ Since the 5-LO pathway is activated in many inflammatory diseases but is likely resistant to corticosteroid treatment,²⁰,²⁹-³⁴ inhibition of the LTB₄-dependent pathway by modulating either LTB₄ synthesis (inhibition of cPLA₂, 5-LO or LTA₄ hydrolase), the effects of LTB₄ itself (LTB₄ receptor antagonism), or degradation of LTB₄ by peroxisome proliferator-activated re
ceptron α (PPARα) activator may have important benefits in the treatment of these allergic diseases.

**BIOSYNTHESIS AND DEGRADATION OF LTB4**

LTB₄ is not stored and released but synthesized de novo from arachidonic acid in activated innate immune cells such as granulocytes, macrophages, and mast cells following several enzymatic steps. Arachidonic acid is released from membrane phospholipids by the action of cPLA2 after the activation of cells by mechanical injury, infection, allergens, cytokines, or growth factors. Following cellular activation, 5-LO is translocated to the nuclear membrane and then receives arachidonic acid donated by the integral nuclear membrane protein termed 5-LO-activating protein (FLAP). 5-LO converts arachidonic acid to 5-hydroperoxycisotaetraenoic acid (5-HPETE) and then the unstable intermediate LTA₄. LTA₄ is further metabolized to either LTB₄ by LTA₄ hydrolase or CysLTs by LTC₄ synthase. Since 5-LO expression is restricted to myeloid cells while LTA₄ hydrolase and LTC₄ synthase are expressed widely, LTs are mainly produced by leukocytes. Neutrophils produce LTB₄ which is strongly chemotactic for leukocytes, whereas the CysLTs produced by eosinophils increase the contractility of vascular and airway smooth muscle cells. Monocytes/macrophages are also able to produce both of these LTs.

LTB₄ is inactivated through metabolic degradation by the microsomal ω-oxidation, mitochondrial and peroxisomal β-oxidation pathways. In the ω-oxidation pathway, LTB₄ is converted to 20-hydroxy LTB₄ by LTB₄ ω-hydroxylases, which belong to the cytochrome P450 family 4F3 (CYP4F3) and then to 20-carboxy LTB₄ by LTB₄ ω-hydroxylases in neutrophils or alcohol dehydrogenase and aldehyde dehydrogenase in hepatocytes. Two isozymes, CYP4F3A and CYP4F3B, are expressed in granulocytes and liver, respectively. Since LTB₄ is a hydroxyl fatty acid, peroxisomal and mitochondrial β-oxidation can be also involved in the degradation of LTB₄. The 12-hydroxyicosanoid dehydrogenase expressed ubiquitously can inactivate LTB₄ to 12-keto LTB₄.

**LTB₄ RECEPTORS AND SIGNAL TRANSDUCTION**

Three distinct receptors for LTB₄ have been identified. The PPARα is the nuclear receptor for eicosanoids including LTB₄ and their interaction promotes degradation of lipid mediators. PPARα binds to PPAR responsive elements (PPREs) at the promoter sites of several lipid metabolism-related enzymes such as LTB₄ ω-hydroxylases (CYP4F3). LTB₄ binds and activates PPARα, resulting in the transcription of genes that promote fatty acid degradation. PPARα-deficient mice showed prolonged inflammatory responses in arachidonic acid-induced ear swelling. Moreover, PPARα activator can inhibit arachidonic acid-induced murine ear inflammation by enhancing the degradation of LTB₄. These data suggest that PPARα plays an important role in the clearance of lipid mediators during inflammation.

Other receptors are cell surface receptors for LTB₄. Both BLT1 and BLT2 are the G protein-coupled seven transmembrane domain receptors for LTB₄, and their coding genes are located in very close proximity to each other in the human or mouse genomes. These receptors differ in their affinity and specificity for LTB₄ and their expression pattern. BLT1, a specific high affinity receptor for LTB₄, is expressed predominantly on leukocytes including granulocytes, monocytes/macrophages, mast cells, dendritic cells, and effector T cells whereas BLT2, a low affinity receptor which can also bind to other eicosanoids, is expressed ubiquitously and their biological role in humans is unknown. Mouse BLT2 is expressed highly in small intestine and skin and a BLT2-selective agonist induced chemotaxis in primary mouse keratinocytes and bone marrow mast cells. Ligation of BLT1 and/or BLT2 by LTB₄ triggers a variety of intracellular signal transduction and cellular events in inflammatory cells, including intracellular Ca²⁺ mobilization, activation of extracellular signal-regulated kinase 1/2, phosphoinositide-3 kinase and Akt, chemotaxis, degranulation, and/or the production of inflammatory proteins.

**ASTHMA AND LTB₄**

Asthma is a complex and heterogeneous chronic disease characterized by reversible airway obstruction, allergic airway inflammation, and airway hyperresponsiveness (AHR). LTB₄ levels were increased in the sputum, plasma, and bronchoalveolar lavage (BAL) fluid of asthmatic patients but not of healthy subjects. Increased synthesis of LTB₄ was accompanied by increased transcriptional upregulation of 5-LO and LTA₄ hydrolase in peripheral blood leukocytes of asthmatic children. Generation of LTB₄ by calcium ionophore-stimulated alveolar macrophages and peripheral blood neutrophils was increased in asthmatic patients compared to those from healthy subjects. These data suggest that an upregulation of the LTB₄ synthetic pathway in the circulating leukocytes and lungs is associated with asthma.

LTB₄ is associated with development of AHR as well as during an asthma attack. Inhaled methacholine, which is widely used to evaluate AHR, stimulates LTB₄ release in patients with asthma, but not in healthy subjects, without affecting the number of inflammatory cells in BAL fluid. LTB₄ was increased in the arterial blood of asthmatic patients during wheezing attacks. LTB₄ levels in BAL fluid were significantly increased in nocturnal asthma and correlated with the nocturnal fall in forced expiratory volume in one second (FEV₁). Zileuton (a 5-LO in-
hibitor) or corticosteroid treatment decreased LTB4 levels and eosinophil numbers in BAL fluid and improved FEV1 in patients with nocturnal asthma.\textsuperscript{46,47} Zileuton blocked allergic airway inflammation and AHR in sensitized and challenged animals\textsuperscript{48} and decreased AHR to cold dry air in patients with moderate asthma,\textsuperscript{49} suggesting that 5-LO metabolites contribute to the pathogenesis of asthma. A clinical trial with the LTB4 antagonist, LY293111, in allergen-induced asthmatic responses in 12 atopic patients with mild intermittent asthma failed to show a therapeutic benefit except for an inhibitory effect on neutrophil recruitment, but no evidence was presented confirming activity \textit{in vivo} or duration of action.\textsuperscript{50} LY293111 prevented development of airway hyperresponsiveness and neutrophil accumulation but failed to inhibit accumulation of eosinophils in sensitized and challenged animals.\textsuperscript{51} However, recent studies using another BLT1 antagonist (CP-105,696) and BLT1-deficient mice have confirmed the important, if not essential, role of the LTB4/BLT1 pathway in the recruitment of not only neutrophils but also effector T cells including effector memory CD8+ T cells into the lungs of allergen-induced allergic airway inflammatory responses in mice.\textsuperscript{52-58} Inhibition of the LTB4/BLT1 pathway resulted in decreases in airway hyperresponsiveness and allergic airway inflammation including accumulation of eosinophils and lymphocytes in the airway. Similar to the mouse model of allergic inflammation, BLT1-expressing effector memory CD8+ T cells are increased in BAL fluid of human asthmatics.\textsuperscript{8,9} Although further clinical studies are needed to evaluate the role of LTB4 in human asthma, the LTB4/BLT1 pathway appears to play an important role in the pathophysiology of asthma, likely in conjunction with other mediators including CysLTs, cytokines, and chemokines.

**EXERCISE-INDUCED ASTHMA (EIA) AND LTB4**

EIA represents airway narrowing that occurs in association with exercise. Several studies demonstrated a causal relationship between LTB4 and EIA. Zymosan- or calcium ionophore-stimulated LTB4 production by peripheral blood neutrophils was increased after development of EIA.\textsuperscript{50} Recently, it was shown that anaerobic exercise-induced stress can enhance the transcription of genes such as \textit{ALOX5} and \textit{ALOX5AP}, which encode for 5-LO and FLAP in leukocytes and thus increase plasma LTB4 and LTC4 levels even in healthy subjects,\textsuperscript{27} suggesting that LTB4 and CysLTs may play a role in EIA. Indeed, 5-LO inhibitors have been shown to attenuate EIA.\textsuperscript{7} Fish oil-enriched supplements [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] reduced severity of EIA and plasma LTB4 and LTE4 levels,\textsuperscript{60} suggesting fish oil supplementation may represent a potentially beneficial non-pharmacologic intervention for EIA patients.

**ASPIRIN-SENSITIVE ASTHMA (ASA) AND LTB4**

ASA is characterized by the triad of aspirin sensitivity, severe asthma, severe chronic rhinosinusitis and recurrent nasal polyposis. ASA is more commonly found in non-atopic, middle-aged female asthmatic patients. Increased levels of LTB4 and CysLTs in nasal polyps have been reported in patients with ASA.\textsuperscript{61} Furthermore, LTB4 release, but not LTC4, by calcium ionophore-stimulated peripheral blood monocytes was increased after oral aspirin challenge and decreased after aspirin desensitization in ASA patients,\textsuperscript{28} suggesting a key role for LTB4 in the pathogenesis of ASA. However, other data demonstrated conflicting results that LTC4 and its receptor CysLT1, but not the LTB4/BLT1 pathway, is involved in the pathogenesis of aspirin sensitivity\textsuperscript{62} and anti-CysLTs agents may be the preferred adjunctive therapy for ASA.

**ALLERGIC RHINITIS AND LTB4**

LTB4 is closely related to the pathogenesis of allergic rhinitis. Specific allergen challenge provokes significant increases in nasal airway resistance, numbers of neutrophils and eosinophils, and levels of protein, histamine, LTB4, and CysLTs in nasal lavage fluid from patients with allergic rhinitis.\textsuperscript{10,11} Peripheral blood neutrophils from patients with allergic rhinitis produced more LTB4 after calcium ionophore stimulation than healthy subjects.\textsuperscript{63} Allergen-induced nasal congestion and increased LTB4 levels in nasal lavage fluid of patients with allergic rhinitis were significantly decreased by treatment with zileuton.\textsuperscript{12} Selective histamine receptor H1 antagonists also decreased LTB4 levels in nasal fluid from patients with allergic rhinitis.\textsuperscript{13} Although the mechanism of action of currently used antihistamines is primarily through competitive antagonism of H1 receptors on nerve endings, smooth muscles, and glandular cells, H1 receptor antagonism is not likely the sole mechanism of their action in the treatment of allergic diseases. Based on \textit{in vitro} and animal experiments, H1 receptor antagonists also have additional pharmacological properties such as inhibiting LTs release.\textsuperscript{64} However, LTB4 levels in nasal lavage were not changed after topical corticosteroid treatment even with marked reduction in nasal symptoms, levels of histamine, cytokines, and numbers of eosinophils.\textsuperscript{33,34}

**ATOPIC DERMATITIS AND LTB4**

Atopic dermatitis is a chronic, relapsing allergic skin disease that affects over 2% of the population. The pathophysiology of atopic dermatitis is not completely determined, but immunologic abnormalities and the subsequent release of inflammatory mediators might play a central role. Topical corticosteroids have been the gold standard of treatment for a long
time. Many reports demonstrated increased levels of LTB4 in skin lesions with atopic dermatitis or allergic contact dermatitis.14-16 Spontaneous and C5a, anti-IgE, or calcium ionophore-stimulated LTB4 release by peripheral blood neutrophils was enhanced in patients with atopic dermatitis.65 The enzymatic activities of LTA4 hydrolase in peripheral blood leukocytes were associated with disease severity in patients with atopic dermatitis and were reduced after improvement of the disease.66 Since increased levels of LTB4 were also demonstrated in other skin diseases such as psoriasis,14 increases in LTB4 levels do not appear to be a specific feature of atopic dermatitis. Taken together, these facts and the biological effects of LTB4 implicate their involvement in the pathogenesis of cutaneous inflammation in atopic dermatitis, and may provide a new target for pharmacological treatment of this disease.

ALLERGIC CONJUNCTIVITIS AND LTB4

LTB4 causes eosinophil and neutrophil emigration into conjunctival tissue.67 LTB4 levels were increased in the tears of patients with seasonal allergic conjunctivitis17 and were decreased by topical treatment with drugs such as disodium cromoglycate, lodoxamide, or fluorometholone,18 suggesting a role for LTB4 in the pathogenesis of allergic conjunctivitis. The role of LTB4 and its receptor in the pathogenesis of allergic conjunctivitis and potential benefits of LTB4-specific antagonists in the treatment of allergic conjunctivitis remain to be defined.

FISH OIL FATTY ACIDS AND LTB4 IN ALLERGIC DISEASES

Fish oils contain 20- and 22-carbon n-3 fatty acid, EPA and DHA. Since EPA and DHA are incorporated into cell membranes after dietary intake of fish oils and inhibit 5-LO competitively, they can reduce the content of arachidonic acid and thus suppress conversion of arachidonic acid to LTB4.68 Furthermore, it has been recently shown that resolvin E1 (5S, 12R, 18R-trihydroxyeycosapentaenoic acid), an anti-inflammatory lipid mediator derived from EPA, binds and blocks BLT1 and is involved in resolution of allergic inflammation.69 Indeed, ingestion of fish oils changed the fatty acid composition of neutrophils, decreased LTB4 generation by neutrophils and monocytes, and reduced chemotactic activity of neutrophils in asthmatic patients.70 Moreover, fish oils-enriched supplementation attenuated allergen-induced late asthmatic responses in atopic asthma71 and reduced the severity of EIA and plasma LTB4 levels.72 These findings suggested that fish oil supplementation may represent a potentially beneficial nonpharmacologic intervention for LTB4-dependent allergic diseases.

CORTICOSTEROIDS AND THE LTB4/BLT1 PATHWAY IN ALLERGIC DISEASES

Corticosteroids effectively suppress inflammatory responses through repression of many immune genes by interaction with the glucocorticoid receptor and are therefore widely used for the treatment of various allergic diseases. Th2 cytokine-producing CD4+ T cells and eosinophils play important roles in the pathogenesis of allergic diseases, and numbers of these cells, but not CD8+ T cells, were dramatically decreased in peripheral blood, inflamed bronchial tissues or skin of patients with asthma or atopic dermatitis after initiation of corticosteroid treatment.72,73 However, it has been shown that a proportion of asthmatic patients suffered a decline in lung function despite high doses of inhaled or oral corticosteroid treatment.74 A prospective study has shown a significant correlation between annual fall in post-bronchodilator FEV1 and numbers of peribronchial CD8+ T cells, but not eosinophils, CD4+ T cells, mast cells, or subepithelial reticular thickness, in bronchial biopsies of asthmatic patients treated mostly with inhaled corticosteroids.75 We identified CD8+BLT1+IL-13+ T cells in BAL fluid and lung tissues from asthmatic subjects but not in lung tissue or BAL fluid from control subjects.9 Paradoxical negative effects of corticosteroids on neutrophils and monocytes such as increased LTB4-induced chemotaxis and enhanced survival through upregulation of BLT1 expression have been reported.76,77 We recently found that BLT1-expressing effector memory CD8+ T cells are more resistant to corticosteroids than CD4+ T cells and that corticosteroids can enhance the activation and effector function of this CD8+ T cell subset by upregulating BLT1 expression through increased IL-2 receptor expression. This upregulation of BLT1 on effector memory CD8+ T cells by corticosteroids contributes to their ability to enhance the development of AHR, allergic airway inflammation, and goblet cell metaplasia in allergen-sensitized and challenged mice.42 LTB4 production is itself resistant to corticosteroid treatment.20,29-34 Corticosteroid treatment did not reduce the increased LTB4 levels in serum, nasal wash fluid, or BAL fluid from patients with asthma, COPD, or allergic rhinitis.20,30,31 and did not inhibit calcium ionophore-stimulated release of LTB4 by alveolar macrophages obtained from wheezing infants.32 Furthermore, a neutrophil-activating factor which augments LTB4 generation by human neutrophils was identified in peripheral blood monocytes isolated from corticosteroid-resistant asthmatics.29 Although some reports showed conflicting results with decreased in vitro generation of LTB4 by alveolar macrophages after corticosteroid treatment31,46 cumulatively these findings suggest that the corticosteroid-resistant LTB4/BLT1/CD8+ T cell pathway may contribute to the pathogenesis of
corticosteroid-insensitive allergic inflammation (Fig. 1).

CONCLUSIONS

Although approximately 30 years have past since LTB₄ was discovered in 1979, recent progress in research using BLT1 antagonists and BLT1-deficient mice have revealed the important roles of the LTB₄/BLT1 pathway in the pathophysiology of various allergic diseases. It is now recognized that corticosteroid administration in chronic severe persistent allergic diseases may not be disease-modifying and ineffective in preventing or reversing the progression of disease in certain patients. Targeting of the LTB₄/BLT1 pathway may be an important additive to the treatment of such patients with “corticosteroid-insensitive” allergic diseases.

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LTB₄ in Allergic Diseases


