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Review

Too much of a good thing: How modulating LTB_4 actions restore host defense in homeostasis or disease

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ABSTRACT

The ability to regulate inflammatory pathways and host defense mechanisms is critical for maintaining homeostasis and responding to infections and tissue injury. While unbalanced inflammation is detrimental to the host; inadequate inflammation might not provide effective signals required to eliminate pathogens. On the other hand, aberrant inflammation could result in organ damage and impair host defense. The lipid mediator leukotriene B_4 (LTB_4) is a potent neutrophil chemoattractant and recently, its role as a dominant molecule that amplifies many arms of phagocyte antimicrobial effector function has been unveiled. However, excessive LTB_4 production contributes to disease severity in chronic inflammatory diseases such as diabetes and arthritis, which could potentially be involved in poor host defense in these groups of patients. In this review we discuss the cellular and molecular programs elicited during LTB_4 production and actions on innate immunity host defense mechanisms as well as potential therapeutic strategies to improve host defense.

1. Introduction

The ability of innate immune cells to properly recognize, respond, and eliminate invading pathogens is a requisite for host survival. Microbial infections quickly elicit an inflammatory program that induces the recruitment of phagocytes, such as macrophages, monocytes and neutrophils. These newly migrated cells further enhance the production of pro-inflammatory mediators such as cytokines, chemokines, growth factors, and bioactive lipids in the inflammatory milieu. After exposure to pathogens, sentinel cells such as epithelial cells and phagocytes (tissue resident macrophages and dendritic cells) detect pathogen associated molecular patterns (PAMPs) via binding to pattern recognition receptors (PRRs). Activation of PRRs such as Toll like receptors (TLRs) trigger the signaling programs that culminate in the generation of inflammatory cytokines and lipid mediators to provide signals essential to the recruitment of cells involved in the control of pathogens. The bioactive lipid mediator leukotriene B_4 (LTB_4) is produced primarily by neutrophils and macrophages and signals through its high or low affinity receptor B leukotriene receptor (BLT) 1 or 2, respectively, to enhance phagocyte antimicrobial effector functions. However, aberrant levels of LTB_4 can be detrimental to host response and may be pathogenic in inflammatory diseases.

Effective inflammatory programs induced during infections can be compromised by underlying health conditions [1]. Although inflammation is important for coordinating immune responses during infection, excessive inflammatory responses can be destructive. Patients with chronic inflammatory diseases, such as diabetes, arthritis, and atherosclerosis have dysregulated inflammatory response functions and are more prone to infections [2–4].

This review covers the current understanding of the role of LTB_4 and BLT1 on host defense mechanisms and how modulating the LTB_4/BLT1 pathway can be therapeutically targeted to respectively amplify or inhibit host defense in settings of immunodeficiency or aberrant inflammation.

2. LT synthesis and receptors

2.1. LT synthesis

LTs are part of a large family of lipids termed eicosanoids that are derived from the Greek word “eicosa” since eicosanoids are made of 20 carbon atoms called eicosatetraenoïc acid. The synthesis of LTs involves

Abbreviations: LT, leukotriene; PAMP, pathogen associated molecular patterns; PRR, pattern recognition receptor; AA, arachidonic acid; LO, lipoxygenase; $cPLA_2$, cytosolic phospholipase A_2; FLAP, 5-lipoxygenase-activating protein; cysLT, cysteinyl LT; BLT, B leukotriene receptor; cAMP, cyclic adenosine monophosphate; PKC, protein kinase C; ERK, extracellular signal-related kinase; PI3K, phosphoinositide 3-kinase; NFκB, nuclear factor kappa B; AP1, activator protein 1

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Table 1
Expression of LT synthesis enzymes and LTB_4 receptors in immune and structural cells.

Cell type	5-LO	FLAP	LTA_4 hydrolase	LTC_4 synthase	BLT1	BLT2
Neutrophil	+ + +	+	+ + +	±	+	+
Monocyte/ macrophage	+ +	+	+ + +	+	+	+
Dendritic cell	+	+	+ +	+	+	+
Mast cell	+	+	+	+ +	+	+
Eosinophil	+	+	+	+ +	+	+
Endothelial cell	−	−	+	+	+	+
Red blood cell	−	−	+	−	−	−
Keratinocyte	±	−	+	+	+	+

several rate-limiting steps that comprise activation of phospholipase A_2 (PLA_2) and arachidonic acid (AA) release from phospholipids in the cellular membranes. Activation of 5-LO in concert with the 5-LO activation protein (FLAP) metabolizes AA to LTA_4, which is converted to LTB_4 by LTA_4 hydrolase. LTA_4 could alternatively be modified with glutathione by LTC_4 synthase to form LTC_4. Further modifications of LTC_4 give rise to LTD_4 and LTE_4. Since LTC_4, D_4, and E_4 contain a cysteine, they are known as the cysteinyl leukotrienes (CysLTs). Even though CysLTs exert stimulatory effects on macrophages and neutrophils, this review will focus primarily on LTB_4 actions. The main cellular sources of LTB_4 in both murine and humans are granulocytes, monocytes, and macrophages (Table 1) [5]. However, murine (RAW264.7 and J774) and human (THP1 and U937) macrophage cell lines express low levels of 5-LO and produce barely detectable levels of LTB_4 [6]. Non-immune cells are also capable to produce LTB_4. Some cell types have been reported to express some but not all of the LT-synthesis enzymes, which renders these cells incapable of synthesizing leukotrienes independently. However, these cells may be able to contribute to the synthesis of LTs in a process known as transcellular biosynthesis [7]. An example of transcellular biosynthesis of LTB_4 is between neutrophils-erythrocytes, and keratinocytes and endothelial cells [8–12]. Table 1 lists the cellular expression of leukotriene synthesis enzymes. Although this topic is of interest, a more comprehensive review can be found at [13].

5-LO activity is dependent on various signals including calcium release and phosphorylation, which control the catalytic site and the translocation of 5-LO within the cell. In a resting cell, 5-LO location varies depending on the cell type [14]. In neutrophils and peritoneal macrophages, 5-LO is located in the cytosol whereas in alveolar macrophages and Langerhans cells, 5-LO is located within in the nucleus [15–17]. Upon cell activation, increased intracellular calcium levels induce 5-LO to translocate to the perinuclear or plasmatic membrane where it can metabolize AA into LTA_4 [18]. 5-LO activity is triggered by various stimuli such as pathogens, cytokines, and immune complexes [19]. During infection, pathogens have limited abilities to increase intracellular calcium levels and therefore are poor 5-LO activators alone. However, treating infected cells with a calcium ionophore or opsonized zymosan particles are able to greatly enhance LT synthesis [20,21]. Table 2 lists the relative levels of LTB_4 produced in response to various stimuli. The molecular mechanisms that regulate 5-lipoxygenase activation are reviewed here [22,23].

Table 2
LTB_4 generation in response to various stimuli. ND not determined.

Cell type	Cytokines/ Growth factors	Bacteria	Opsonized pathogen	Fungi	Viral
Neutrophil	+ +	+ +	+ + +	+ +	+ +
Monocyte/ macrophage	+ +	+ +	+ + +	+ +	+ +
Dendritic cell	+	+ +	ND	+	+
Mast cell	+	+	ND	+	+
Endothelial cell	±	±	±	±	±

2.2. LTB_4 receptors

There are two G protein coupled receptors (GPCRs) for LTB_4, BLT1 and BLT2. BLT1 is a high-affinity receptor and BLT2 is a low-affinity receptor. Since other fatty acid metabolites besides LTB_4 are able to activate BLT2, the effects of BLT2 signaling are not limited strictly to LTB_4 effects [24]. Distribution of BLT1 and BLT2 on cells and tissues vary between mouse and human [24]. On human cells, BLT1 expression is limited to leukocytes (Table 1) and BLT2 is ubiquitously found on many cell types. On mouse cells, BLT1 expression is detected on leukocytes and BLT2 is found on intestinal epithelium and keratinocytes [24,25]. BLT1 can be coupled to Gαi or Gαq that culminate to decrease cyclic AMP (cAMP) levels and increase intracellular calcium levels, respectively [26]. We have previously shown that BLT1 utilizes mainly Gαi to enhance antimicrobial effector functions in alveolar macrophages [26]. Fig. 1 demonstrates a schematic of LTB_4/BLT1-induced effector functions in innate immune cells.

The low affinity receptor BLT2 is also expressed in phagocytes, but its role in host defense is poorly studied. Previously, we have shown blocking BLT2 does not influence phagocytosis and bacterial killing in alveolar macrophages [27]. However, BLT2 might be a relevant receptor for other antimicrobial effector functions in different organs [24]. Recently, Zhang and Brown have shown that in the absence of BLT1, BLT2 enhances macrophage *Borrelia burgdorferi* phagocytosis. It remains to be determined whether BLT2 amplifies neutrophil chemotaxis to the site of infection and whether BLT2 controls antimicrobial peptide production in the skin or in the gut. However, BLT2 regulation and actions are out of the scope of this review.

3. Effects of LTB_4 on host defense mechanisms

3.1. Migration and chemotaxis

Neutrophils are the first cells recruited to the site of infection or injury. There are various steps involved in successful recruitment of neutrophils: rolling, adhesion, and transendothelial migration [28]. LTB_4 is well known for its role as a neutrophil chemoattractant from distant sites to the site of inflammation [29–31]. Also, LTB_4 participates with chemokine gradients to further enhance neutrophil chemotaxis towards other chemoattractants such as fMLP, C5a, and heme [32–34]. Neutrophils from mice lacking BLT1 are not able to swarm and cluster to a focal damage site. $BLT1^{-/-}$ neutrophils have smaller neutrophil clusters than wild type neutrophils [35], demonstrating the importance of LTB_4/BLT1 signaling in neutrophil accumulation. Additionally, neutrophil recruitment is not unidirectional. Reverse transendothelial migration has been reported where neutrophils reenter the blood stream after migration to the site of infection or injury [36]. In a model of ischemia-reperfusion injury, LTB_4 and neutrophil elastase compose an important axis that drives reverse transendothelial migration. Neutrophils that reenter the vasculature are able to migrate to secondary organs and have the potential to cause injury [37].

3.2. Phagocytosis

We, and others, have shown that LTB_4/BLT1 signaling amplifies the actions of different signaling components required for ingestion of particles. The first demonstration that LTB_4 enhances phagocytosis was shown by Wirth et al. using a model of *Trypanosoma cruzi* infection [38]. After this, Dr. Peters-Golden's group pioneered in demonstrating the role of endogenous LTs in amplifying phagocytosis of antibody-opsonized targets [39,40]. Both genetic and pharmacologic blockage of LTB_4/BLT1 actions greatly reduces ingestion of a myriad of pathogens, including both gram-positive bacteria (*Streptococcus pneumoniae*) [27], gram-negative bacteria (*Klebsiella pneumoniae* [41]), fungi (*Histoplasma*