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Leukotriene B_4, an Endogenous Stimulator of the Innate Immune Response against Pathogens

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Leukotriene B_4 · Neutrophils · Toll-like receptor · Antimicrobial peptides · Host defense · Viral infection · Macrophages

Abstract
Leukotriene B_4 (LTB_4) is an endogenous lipid mediator of inflammation derived from arachidonic acid by the sequential action of cytosolic phospholipase A_2 and 5-lipoxygenase. This mediator was initially recognized for its involvement in the recruitment of neutrophils. However, in the last decade, LTB_4 has been clearly demonstrated to play a significant role in the control of microbial infections through its ability to activate host innate defenses. In this review, we will focus on the modulator effects of LTB_4 on the innate defenses and discuss its therapeutic potential against viral pathogens.

Introduction

Leukotrienes (LTs) are a family of mediators known to exert a multitude of biologic effects that contribute to enhance the inflammatory response. LTs are generated from the liberation of arachidonic acid through the action of cytosolic phospholipase A_2 and 5-lipoxygenase (5-LO), which is expressed in all cells of myeloid origin. Upon an increase in intracellular calcium, 5-LO translocates to the nuclear membrane, which requires the action of the membrane anchor 5-LO-activating protein [1]. This process gives rise to the formation of LTA_4, which is converted to LTB_4 through the action of LTA_4 hydrolase [2]. LTA_4 can also be converted to LTC_4 by LTC_4 synthase and constitutes the precursor of other cysteinyl LTs named LTD_4 and LTE_4.

First identified in neutrophils following incubation with arachidonic acid [3], LTB_4 was also shown to be produced by other cell types. In fact, synthesis of LTB_4 has been demonstrated in human alveolar macrophages stimulated with zymosan or ionophore A23187 [4, 5]. Bronchial epithelial cells and fibroblasts are also able to generate LTB_4 [6, 7], indicating that these cells may contribute to the inflammatory response in lungs. Peritoneal macrophages, monocytes and dendritic cells may produce LTB_4 in response to several stimuli including N-formyl-methionyl-leucyl-phenylalanine peptide, zymosan particles, complement complex, bacterial components and endotoxins [8–10].

LTB_4 signaling is achieved through BLT receptors belonging to the G protein-coupled receptor family. In mammalian cells, two LTB_4 receptors are expressed, i.e. the high-affinity BLT1 receptor and the low-affinity BLT2 receptor. BLT1 is mainly expressed by leukocytes, par-

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ticularly neutrophils and eosinophils, whereas BLT2 is expressed ubiquitously, with increased expression in lymphoid organs [11]. The chemoattractive properties of LTB_4 as well as its proinflammatory effects are mainly mediated by the BLT1 receptor. Following binding to its receptor, an increase in Ca^{2+} mobilization is observed and downstream signaling molecules are activated. LTB_4 activates several kinase cascades leading to the transcription of cytokine genes. Two important but distinct signaling pathways are sensitive to LTB_4 action, one leading to the translocation of nuclear factor (NF)-κB, while the other activates members of the mitogen-activated protein kinase (MAPK) family such as p38, c-JUN kinase and extracellular signal-regulated kinases 1/2, leading to the activation of the transcription factor activated protein-1 [12–14]. In addition, LTB_4 was shown to induce neutrophil activation and degranulation through its effects on the β isoform of phosphoinositide 3-kinase and the Src kinase Yes, respectively [15, 16]. LTB_4 activates the GTPase Rac and Rac-extracellular signal-regulated kinase, two signaling molecules that contribute to phagocytosis and chemotaxis [17]. These studies demonstrate that LTB_4 can influence several kinases involved in different downstream signaling pathways associated with the aforementioned biological processes. Therefore, it is not surprising that LTB_4 can exert pleiotropic actions on host cells.

LTB_4 and the Inflammatory Response

Inflammation is a complex physiological response initiated following pathogen recognition and tissue injuries. In the early phase of inflammation, activation of resident macrophages and other immune cells induces the release of cytokines and chemokines that subsequently recruit inflammatory cells to the tissue. During infection, inflammation further correlates with increased transport of pathogens and antigen-presenting cells from the infected tissue to the lymphoid organs. This gives rise to lymphocyte activation and initiation of adaptive immunity characterized by antibody production and effector cell recruitment to the site of infection.

The early phase of the immune response is characterized, as mentioned above, by the rapid recruitment of effector cells and the release of cellular mediators that trigger the onset of the immune response. In this process, LTB_4 acts as a mediator that exerts a central role on inflammatory cells through its chemoattractant properties. This LT was initially recognized as a cellular chemoattractant released from polymorphonuclear leukocytes [18]. This was based on studies showing that intravenous injections of LTB_4 in rabbits resulted in a prompt increase in blood neutrophils, and intradermal injection of LTB_4 leads to an accumulation of neutrophils at the site of injection [19]. The capacity of LTB_4 to recruit effector cells is not limited to neutrophils. The chemoattractant activity of LTB_4 was also demonstrated for monocytes, macrophages, dendritic cells, eosinophils and effector T cells [20–22]. Of potential importance, LTB_4 induces recruitment of CD4 and CD8 effector T cells as well as T helper type 1 and T helper type 2 cells to inflamed tissue [23, 24], providing a link between innate and adaptive immune responses. Early recruitment of activated immune cells during the onset of inflammation constitutes one of the main mechanisms through which LTB_4 modulates the inflammatory process.

Following their entry into peripheral tissue, the recruited immune cells will engage in the inflammation process by secreting cytokines and chemokines, which also contribute to promote inflammation. Evidence shows that LTB_4 is active in this process and enhances the production of several inflammatory mediators such as interleukin (IL)-2 and IL-5 by T cells. LTB_4 was also found to increase the sensitivity of T lymphocytes to IL-2, a mediator essential for T cell proliferation. In fact, in vitro assays showed that lymphocytes that had been incubated with LTB_4 for 24 h were more responsive to IL-2, showing that LTB_4 increased the sensitivity of cytotoxic lymphocytes to IL-2 activation [25]. This increased activity was attributed to an upregulation of IL-2 receptor β expression, which is mostly expressed on natural killer and CD8 T cells. Similarly, LTB_4 induces rapid, dose-dependent IL-5 production in T cells [26]. These cytokines are produced by T helper type 1 and type 2 cells and together contribute to activation of the immune response, characterized by the recruitment, proliferation and activation of immune cells.

LTB_4 increases the production of IL-1 (α/β), IL-6 and chemokine (C-C motif) ligand 2 (monocyte chemotactic protein-1) in monocytes and the release of IL-8 by neutrophils. Production of IL-1 by human monocytes stimulated with lipopolysaccharide (LPS) was potentiated in LTB_4-pretreated cells [27]. This mediator is also considered a potent inducer of chemokine (C-C motif) ligand 2 (monocyte chemotactic protein-1) secretion in monocytes [28]. Similarly, LTB_4 induces the release of chemokine (C-X-C motif) ligand 8 (IL-8) by neutrophils and potentiates IL-8 secretion in response to various stimuli [29, 30]. Overall, the role of LTB_4 in the inflammatory re-

sponse can be either directly associated with the recruitment of inflammatory cells or indirectly with its capacity to induce secretion of inflammatory mediators by surrounding cells.

Being associated with various diseases, including chronic pulmonary disorders, drugs targeting LTs were developed as anti-inflammatory medications over the last 20 years [31]. Cysteinyl LT is especially suspected to play a role in asthma, and LTB_4, being recognized as a potent chemoattractant for leukocytes, was also associated with the inflammatory response. Due to the physiological functions it exerts on cells, LTB_4 has long been considered a detrimental mediator of inflammation.

From a clinical perspective, evidence suggests that LTB_4 plays a key role in various inflammatory diseases, especially in lung diseases such as asthma or chronic obstructive pulmonary disease. Increased levels of LTB_4 have been identified in the sputum, plasma and bronchoalveolar lavage fluid of asthmatic patients [32–34]. The levels of LTB_4 were also found to be increased in the exhaled breath condensate of chronic obstructive pulmonary disease patients [35]. Since bronchial epithelial cells and lung fibroblasts are able to secrete LTB_4, it was assumed that in these pathological conditions, LTB_4 could exacerbate inflammatory symptoms through the recruitment and accumulation of inflammatory cells in the respiratory tract along with the secretion of inflammatory cytokines. Nevertheless, new findings on the role of LTB_4 in controlling viral infection and the mechanisms associated with such effects provide new insights into the beneficial role of LTB_4 as a regulator of the innate response.

LTB_4 Is an Activator of the Innate Immune Response

LTB_4 Contributes to the Control of Viral Infection

Release of eicosanoids like LTs is known to influence many biological processes. Their regulatory roles in inflammation and immune defense against viral infections are of particular interest [reviewed in 36], especially with regard to LTB_4, which was reported to exert appealing activity on innate defenses against pathogens. In fact, besides its association with the inflammatory process, several reports from the last decade highlight the involvement of LTB_4 in the activation of innate immune defense against viral infection. The first evidence of the antiviral activity of LTB_4 was demonstrated against Epstein-Barr virus (EBV) and herpes simplex type 1 virus, two viruses of the herpes family [37, 38]. In vitro treatment of EBV-infected human peripheral blood mononuclear cells with LTB_4 was found to strongly reduce the B cell growth-transforming ability of EBV as reflected by the reduced expression of Epstein-Barr nuclear antigen in infected cells. Similar results were also obtained in Vero cell cultures infected with herpes simplex type 1 virus, in which the percentage of infected cells was also affected by the addition of exogenous LTB_4. Interestingly, in these experiments, the antiviral potency of LTB_4 was comparable to the inhibitory effect of acyclovir, a drug widely used for the treatment of herpes infection in humans [37, 38]. These initial experiments clearly demonstrated that treatment with LTB_4 can control viral infection. In addition, treatment of cell cultures with LTB_4 did not block viral entry into the cell, suggesting that it may act on intracellular components to suppress viral replication. More recently, EBV-infected cord blood cell cultures were used to show that LTB_4 contributes to suppress the B cell transformation induced by EBV [39, 40]. The authors demonstrated that inhibition of B cell proliferation by LTB_4 was associated with activation of effector T cells.

The potential role of LTB_4 in viral infection was further demonstrated in vivo in mice infected with murine cytomegalovirus (MCMV) [41]. Intravenous administration of LTB_4 to mice infected with a lethal dose of MCMV significantly reduced the mortality rate as compared to the placebo group. Body weight and temperature data were also in accordance with the survival rate of mice treated with LTB_4. Daily LTB_4 treatment of mice infected with a sublethal dose of MCMV was also found to reduce viral loads titrated in the salivary glands of the mice. Considering that CMV reactivation can be observed in humans, especially in those receiving immunosuppressive therapies, studies aimed at evaluating the protective role of LTB_4 against viral reactivation were also performed. Using an allogenic bone marrow transplantation model, administration of LTB_4 was found to prevent MCMV reactivation in latently infected mice. LTB_4-associated effects were confirmed in 5-LO-deficient mice infected with MCMV and in wild-type infected mice pretreated with MK-886, an inhibitor of 5-LO-activating protein. In these animals, inhibition of LT synthesis results in a significant increase in the viral load in salivary glands, supporting a role for endogenous LTB_4 in the host defense against CMV infection.

The potential efficacy of LTB_4 to activate the host defense against viral infection was also tested with influenza virus, a single-stranded RNA virus. Daily administration of LTB_4 to influenza-infected mice potentiated the reduction of lung viral loads as opposed to mice treated with a placebo [42]. Results from this study indicate that recruit-

ment of neutrophils to the lungs is an important event in controlling influenza infection, which correlates with a decrease in viral load and restored lung architecture. While not specifically identifying LTB_4, another study investigating the role of LTs in acute vesicular stomatitis virus encephalitis documented the protective role of LTs in viral infection [43]. The authors demonstrated that pharmacologic or genetic inactivation of the 5-LO enzyme correlates with a higher viral titer in the brain during early phases of infection. Interestingly, other investigations suggested that impairment of LT synthesis in acquired immunodeficiency syndrome might contribute to the observed susceptibility to opportunistic infections, supporting the beneficial effects of LTs in innate defense against viruses [44, 45]. Recently, it was shown that LTs, including LTB_4 and LTC_4, could inhibit human immunodeficiency virus type 1 (HIV-1) infection in vitro in human monocyte-derived microglia-like cells [46]. LTB_4 was demonstrated to significantly reduce viral loads in this primary cell system, suggesting a protective role in the central nervous system of HIV-infected patients.

On the other hand, it was reported that LTB_4 and LTC_4 were increased in the plasma of human T lymphotropic virus type 1-infected patients. The authors suggest that such an enhanced concentration of LTB_4 and LTC_4 could potentially contribute to the dysregulation of the immune system in neuroinflammatory disease [47]. In a second study, it was shown that human CMV infection of vascular smooth muscle cells leads to enhanced 5-LO expression, resulting in an increase in LTB_4 production [48]. This study proposed that human CMV could initiate and sustain inflammation through the control of 5-LO expression in patients with ulcerative colitis. Despite the fact that, in the specific context of infection, upregulation of the 5-LO pathway may compromise the immune response, LTB_4 is mainly associated with beneficial effects in controlling viral infections.

LTB_4 Is Involved in Antibacterial Immunity

In addition to its potential role in controlling viral infection, LTB_4 was also suggested to contribute to bacterial clearance [reviewed in 49]. The first evidence of the antibacterial role of LTB_4 was highlighted with the demonstration that intraperitoneal administration of LTB_4 resolves experimental bacterial peritonitis in mice [50]. The increased clearance of *Salmonella typhimurium* observed in these animals was proposed to occur through an enhanced bactericidal action of macrophages since this phenomenon was abrogated in animals with high susceptibility to bacterial infection. In mice deficient for the 5-LO gene, it was demonstrated that the host response to pulmonary bacterial infection was mediated by LTs. Following intratracheal *Klebsiella pneumoniae* challenge, a marked increase in LTs and neutrophils was observed in wild-type mice, whereas this response was impaired in knockout mice. This phenomenon correlated with a significant increase in lethality and bacteremia in LT-deficient animals as compared to wild-type mice [51]. The reduced capacity for bacterial clearance of LT-deficient mice was associated with a defect in the phagocytic activity of alveolar macrophages. Interestingly, such defects were overcome in vitro by treatment of macrophages with exogenous LTB_4. Similar conclusions were reached in studies using mice infected with fungal and bacterial organisms and treated with LT synthesis inhibitors, such as MK-886 and A-63162, in which reduced survival rate and bacterial clearance, respectively, were observed [52, 53]. Together, these data indicate that LTs and especially LTB_4 plays a significant role in host defense against microbial pathogens.

Innate Effector Mechanisms Activated by LTB_4 to Control Bacterial and Viral Infection

Destruction of microorganisms requires the contribution of complex intracellular mechanisms such as activation of cytotoxic systems and phagocytosis. The contribution of LTs to the modulation of antimicrobial effector functions and the role of LTB_4 in antibacterial defenses have been documented [49]. Briefly, LTB_4 modulates killing of bacteria through the synthesis of the reactive oxygen intermediate, nitric oxide, and hydrogen peroxide. LTB_4 may also regulate the release of lysosomal enzymes, cytokine production and phagocytosis, which are necessary to neutralize pathogens. These mechanisms activated by LTB_4 are summarized in table 1. Recent insights into LTB_4-activated mechanisms to control both bacterial and viral infections are discussed in the following sections.

LTB_4 Induces Release of Antimicrobial Peptides

LTB_4-induced antimicrobial peptide synthesis has been demonstrated against a diverse range of bacteria [49]. The mechanisms underlying the antiviral activity of LTB_4 are not fully understood and remain to be characterized. However, one of the known effective strategies to fight a wide range of pathogens includes the release of antimicrobial peptides. These antimicrobial peptides are known to exert broad-spectrum activity against bacteria,

Table 1. LTB_4-associated antimicrobial activity on effector cells

Cells	Antimicrobial functions	Microorganisms	References
Neutrophils	nitric oxide		[63, 64][a]
	reactive oxygen species		[65][a]
	β-glucuronidase/lysozyme		[66][a]
	increased phagocytosis	*Klebsiella pneumoniae*	[67][a, b]
	superanion production		[68, 69][a]
	LL-37, EDN, α-defensins	CMV	[55][a]
	myeloperoxidase	CMV	[55][a, b]
		coronavirus, influenza, respiratory syncytial virus	[60][a]
	β-defensin-3, mEARs, CRAMP	influenza	[42][b]
	LL-37, EDN	influenza	[42][a]
	α-defensins, cathepsin G, elastase, lysozyme C, LL-37	herpes simplex virus type 1/HIV	[70][a]
	α-defensin, MIP-1β	HIV	[59][a]
Eosinophils	nitric oxide		[64][a]
	superanion production		[71][b]
	NADPH oxidase activity		[72, 73][b]
Monocytes/ macrophages	nitric oxide	*Trypanosoma cruzi*	[74][b]
	increased phagocytosis	*Trypanosoma cruzi*	[75][b]
		Klebsiella pneumoniae	[51, 76][b]
		group A *Streptococcus*	[77][a, b]
		Salmonella typhimurium, Pseudomonas aeruginosa	[50][b]
	hydrogen peroxide	*Salmonella typhimurium, Pseudomonas aeruginosa*	[50][b]
	NADPH oxidase activity	*Klebsiella pneumoniae*	[78][a]
		group A *Streptococcus*	[77][a, b]
Lymphocytes	immunoglobulin secretion	*Staphylococcus aureus*	[79][a]
	increased CD23 expression		[80][a]
	reduced B cell transformation	EBV	[37][a]
			[40][a]

EDN = Eosinophil-derived neurotoxin; mEARs = mouse eosinophil-associated RNase; CRAMP = cathelicidin-related antimicrobial peptide; MIP-1β = macrophage inflammatory protein-1β; NADPH = nicotinamide adenine dinucleotide phosphate.
[a] Human model. [b] Rodent model.

fungi and enveloped viruses [54]. For instance, β-defensin is known for its antibacterial properties through its membrane disruption effect and is also effective against yeast, such as *Candida albicans*. Cathelicidins are thought to neutralize LPS found on bacteria. Finally, eosinophil-derived neurotoxin and its murine homologue mouse eosinophil-associated RNase are two RNases with antiviral activity against respiratory viruses [42]. In fact, secretion of antimicrobial peptides by neutrophils was found to contribute to the antiviral activities of LTB_4 against influenza virus [42]. Indeed, treatment with LTB_4 leads to a decrease in viral load associated with increased expression of antimicrobial peptides, including β-defensin-3, mouse eosinophil-associated RNase and cathelicidin-related antimicrobial peptide, in lungs of mice infected with influenza virus. In addition, neutrophils were considered to be essential in such production of antimicrobial peptides since in neutrophil-depleted mice, inability of LTB_4 to reduce lung influenza viral loads and induce production of antimicrobial peptides was observed. Similar effector mechanisms could be activated in humans since in vitro treatment of primary human neutrophils with LTB_4 also led to rapid secretion of multiple antimicrobial peptides, which was abrogated with a BLT1 receptor-specific antagonist [42, 55].

The LTB_4-mediated release of antimicrobial peptides was also demonstrated in infection with CMV. Treatment of CMV-infected human peripheral blood leukocytes with LTB_4 led to a reduction of viral titers, a process involving neutrophil activation and release of antimicro-

bial peptides [55]. Similar results were obtained in ex vivo experiments using neutrophils isolated from peritoneal lavage of wild-type and BLT1–/– mice, supporting the role of antimicrobial peptides in anti-CMV effects mediated by LTB_4.

LTB_4 Potentiates Toll-Like Receptor Signaling

An effective immune defense against viruses is initiated following recognition of the viral components by encoded pattern recognition receptors including the Toll-like receptors (TLRs) [56]. Following binding to its receptor, LTB_4 activates signaling molecules interconnected with other cellular receptors such as TLRs. In fact, MAPK- and NF-κB-mediated cytokine production are signaling events common to BLT1 and TLRs. Therefore, we can assume that LTB_4 could influence the cell response to agonists recognized by TLRs. In this regard, Serezani et al. [57] have reported that LTB_4 amplifies NF-κB activation in macrophages stimulated with MyD88-dependent agonists. Macrophages isolated from 5-LO–/– mice showed reduced production of cytokines in response to LPS and peptidoglycans, in addition to reduced p65 binding activity. Similar observations were made with macrophages isolated from BLT1–/– mice, confirming the specific role of LTB_4/BLT1 in MyD88-dependent NF-κB activation. The authors concluded that the enhanced effect induced by LTB_4 on MyD88 expression and cytokine production was mediated by the activation of Janus kinase 2/signal transducer and activator of transcription 1 signaling, suggesting the action of LTB_4 on the MyD88 pathway.

In a more recent study aiming to investigate the interactions of LTB_4 with the TLR system, it was demonstrated that potentiation of cytokine release following stimulation of neutrophils with a combination of LTB_4 and TLR ligands such as lipoteichoic acid, LPS and CpG was dependent on activation of transforming growth factor-β-activated kinase 1 (TAK1), a molecule essential to the MyD88 signaling pathway [29]. LTB_4 potentiation of cytokine secretion in neutrophils stimulated with TLR ligands was suppressed by treatment with TAK1 inhibitor and also in a transfection assay with TAK1-targeting siRNA in HEK293 cells, confirming the action of LTB_4 on TAK1 activity. An interesting aspect of the LTB_4 action on MyD88 signaling is the demonstration that TAK1 and the MAPK p38 are interconnected in LTB_4-treated neutrophils. Indeed, pretreatment of neutrophils with TAK1 inhibitor was found to reduce levels of phosphorylated p38, indicating that activation of p38 by LTB_4 is dependent on TAK1. While not found to be essential, activation of the protein kinase IL-1 receptor-associated kinase 1 (IRAK1; IRAK1/4 complex) by LTB_4 may potentiate cytokine production by neutrophils stimulated with TLR agonists. Since the involvement of TLRs in early recognition of viral particles is crucial to control infection, the overall consequence of such effects of LTB_4 on MyD88 signaling may result in an effective innate response against several types of viruses. A proposed mechanism illustrating the potential interactions between LTB_4 and TLR signaling pathways in neutrophils is presented in figure 1.

Contribution of TLRs to the potentiating effect of LTB_4 on neutrophils was also evaluated. Interestingly, it was demonstrated that LTB_4 differentially modulates the expression of TLRs, depending on whether they are expressed at the cell surface or within the cell [29, 58]. It appears that LTB_4 exerts no effect on expression levels of the membrane receptors TLR2 and TLR4, nor the co-receptors TLR1 and TLR6, on neutrophils. However, LTB_4 can positively upregulate expression of intracellular TLR9 (as well as TLR7/8 mRNA levels), resulting in enhanced binding activity of its ligand. Such a dichotomy remains to be explained, but one might postulate that TLR gene expression could be differentially regulated depending on the cellular origin or the stimulus involved. Together, these results confirm that LTB_4 acts on interconnected signaling molecules to optimize an effective immune response. Moreover, we must also consider that LTB_4 may interact with multiple signaling molecules from different pathways (other than MyD88-dependent pathways) to enhance the state of activation and potentiate immune defenses. This aspect remains to be explored.

Conclusion

Although prostaglandins and LTs are for the most part associated with proinflammatory effects, the studies reviewed herein support the conclusion that LTB_4 benefits the host immune defense. The triggering of the immune response with molecules interacting with the signaling cascade of the innate defense is a very appealing avenue for the development of therapeutic approaches for the treatment of various diseases including viral infections. Investigation of LTB_4 as an 'immunoregulator' is an especially attractive approach for the development of new therapeutics since the available drugs are rather limited and in some cases may present host toxicity.

LTB_4 exerts several biological activities on the immune response, including increasing phagocytosis of microorganisms by neutrophils and macrophages, enhancement

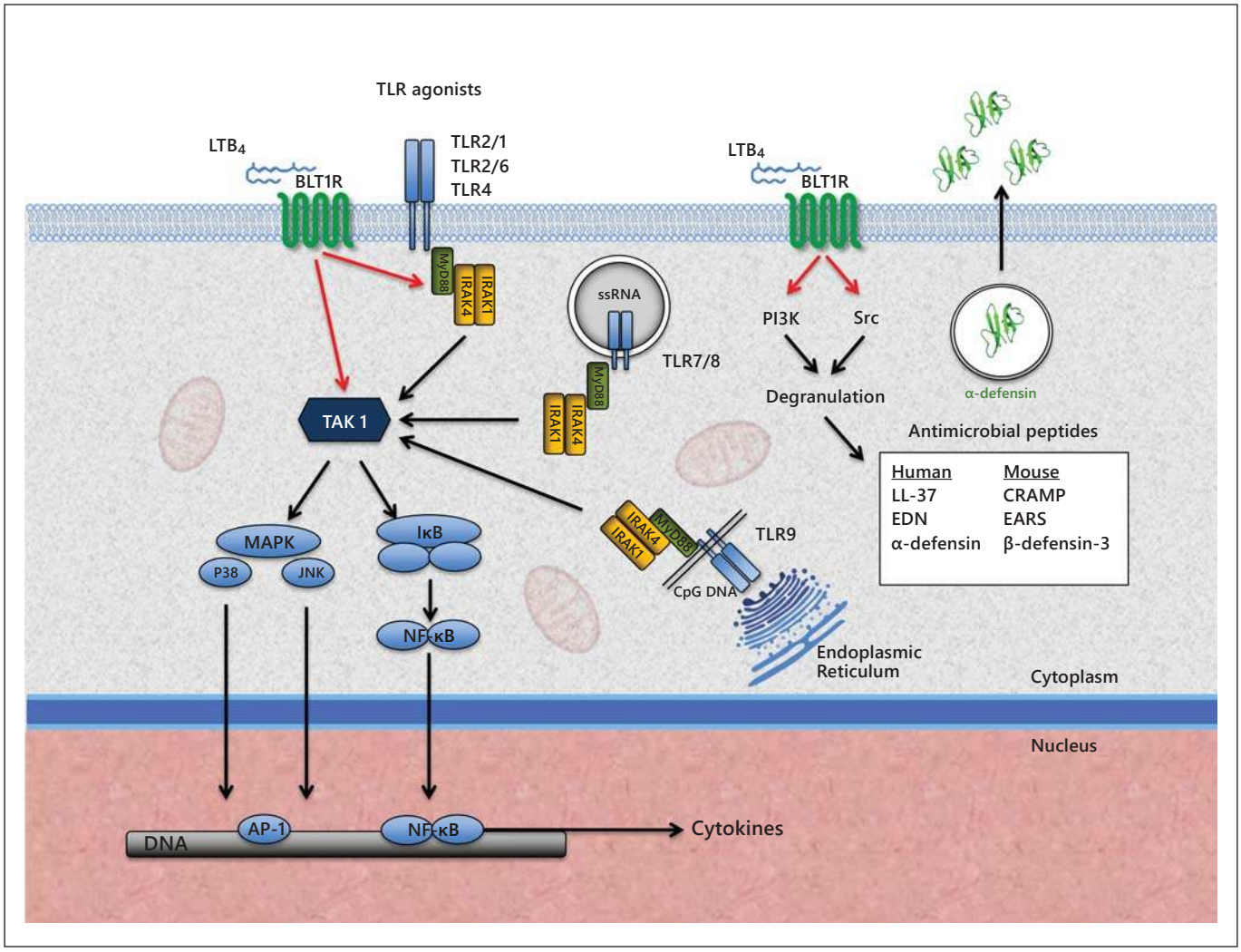

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Fig. 1. Schematic representation illustrating the effects of LTB_4 on the TLR signaling pathway and the release of antimicrobial peptides in neutrophils. With the exception of TLR3, all TLRs lead to the engagement of the MyD88-IRAK1/4-TAK1 axis, which culminates in the activation of MAPK-mediated activated protein-1 (AP-1) production or NF-κB-mediated cytokine production. Upon ligation of LTB_4 to the BLT1 receptor (BLT1R), cellular signaling intersects with downstream TAK1, which occupies a central role in the TLR signaling cascade. In the presence of TLR agonists, LTB_4 potentiates TAK1 activation, leading to an increase in cytokine production. LTB_4 can also activate IRAK1/4, a process which is, however, dispensable to the enhancement of cytokine synthesis. LTB_4 mediates the synthesis and release of antimicrobial peptides [42, 55] through phosphoinositide 3-kinase (PI3K) and Src signaling pathways. Red arrows represent potentiating/activating effects of LTB_4. EDN = Eosinophil-derived neurotoxin; CRAMP = cathelicidin-related antimicrobial peptide; EARS = eosinophil-associated RNase.

of cell recruitment in tissues and production of inflammatory mediators. LTB_4 also induces the release of several antimicrobial peptides by neutrophils. Of particular interest are the interactions of LTB_4 with the TLR system. In fact, by acting on signaling molecules (such as TAK1) at levels shared by different immune receptors, this makes LTB_4 a promising and potentially powerful immune-modulating molecule. Such synergistic activity between signaling cascades could thus contribute to optimize immune defense against pathogens.

Studies in humans clearly indicate that LTB_4 contributes to the regulation of the innate immune response and merits more thorough investigation. Indeed, intravenous administration of LTB_4 to humans was reported to lead to a dose-dependent plasmatic increase in α-defensins [59]. In another study performed in healthy individuals

having received nasal administration of LTB_4, increased production of myeloperoxidase and α-defensins was detected in nasal lavage fluids. In healthy volunteers infected with rhinovirus-16 and treated with LTB_4, reduced viral replication was measured as compared with controls [60]. The impact of LTB_4 on HIV-1-infected patients was also tested [61]. While subjects did not manifest any adverse effects over the trial period, this study failed to show anti-HIV activity. The failure to observe changes in CD4 counts and viral loads might be attributable to the selection criteria of the HIV-infected group. One could consider selecting a more advanced disease state characterized by a higher viral load and a lower CD4 count. In these conditions, subtle improvements in patient status might have been more apparent and translated into higher CD4 counts as well as a reduced viral load. In support of our hypothesis are the results from a previous phase II clinical trial conducted by Boehringer Ingelheim, in which an LTB_4 receptor BLT1 antagonist (BIIL 284 BS) was assessed in the treatment of cystic fibrosis [62]. This multicenter study showed that a significant proportion of adult and pediatric patients treated with this drug experienced adverse events involving pulmonary exacerbation and required hospitalization. The investigators proposed that the high potency of this inhibitor might explain the observed side effects, which probably resulted from a deficient antimicrobial immune response. This highlights the potential of LTB_4 in the regulation of the immune response.

Today, the challenge in the development of antiviral drugs is to design a molecule with high efficacy, low host toxicity and reduced host adverse effects. Moreover, most antiviral agents are designed to treat a specific type of viral infection and usually focus on a virus-associated component. Drugs that boost the immune system without specifically interacting with critical components of the viral life cycle should be considered. This would reduce the selective pressure of virus-associated target therapeutics and would also reduce the appearance of resistant viral strains. Although the antibacterial and antiparasitic properties of LTB_4 seem promising, the future of the potential therapeutic development of this endogenous mediator lies in its antiviral properties and its potential to highlight avenues for new drug development. In addition, we must also consider that if the use of an 'immune booster' like LTB_4 can be controlled to modulate and enhance the level of immune responsiveness, it would constitute a very powerful tool to fight against various microbial infections. LTB_4 could then be used in prophylaxis to prevent infections or in therapy to boost the physiological response against pathogens. Interestingly, this endogenous molecule in combination with other antimicrobial drugs could result in synergic activity that potentiates their therapeutic efficacy.

LTB_4 is an important player in the innate immune defense against infectious microorganisms including viruses. Its 'boosting effects' on the TLR-mediated immune response invite pursuit of the development of such related molecules for the treatment of viral infections and will also be useful in the elaboration of new therapeutic strategies acting on the potentiation of the innate immune response.

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