Microencapsulated Leukotrienes Augment Antimicrobial Activity against Infections

Roberto Nicolete[1,2*] and Larissa DF Nicolete[2]

[1]Laboratório de Imunotecnologia, Centro Universitário do Maranhão (UNICEUMA), Rua Josué Montello, 1, 65075-120, São Luís, Maranhão, Brazil
[2]Fundação Oswaldo Cruz – Fiocruz Rondônia, Rua da Beira 7671, 76812-245, Porto Velho, Rondônia, Brazil

Abstract

Recent evidences support that leukotrienes (LTs) are important molecules in innate and also adaptive immune responses. Some features of these lipid mediators include their ability to be synthesized by a variety of cell types, their diverse antimicrobial actions, and their interactions with other important mediators. More specifically, leukotriene B_4 (LTB$_4$) displays protective effect against infections, such as fungal pneumonia, those caused by helminths and bacterial peritonitis. With respect to histoplasmosis, the fungal infection caused by *Histoplasma capsulatum*, our group has previously demonstrated that these mediators are the main chemoattractants involved in the cell migration induced by the fungus. Regarding new attempts to enhance host antimicrobial response against histoplasmosis, over the last years, we have proposed the employment of a biodegradable microparticulate system, which could release LTB$_4$ to the lungs, as an alternative strategy to treat airway infectious diseases. Here we provide some studies involving the administration of this lipid mediator in several animal and human models of infection and we explore the advantages of the microencapsulated LTB$_4$ as innovative pharmaceutical approach.

Keywords: Leukotriene B_4; Biodegradable microspheres; Infectious diseases; Antimicrobial response

Introduction

Eicosanoids are a group of lipid mediators derived from the fatty arachidonic acid [1]. After release from membrane phospholipids by cytosolic phospholipase A_2 action, arachidonate is further metabolized by the 5-lipoxygenase (5-LO) pathway into leukotrienes (LTs). LTs include both leukotriene B_4 (LTB$_4$) and the cysteinyl LTs (cysLTs) C_4, D_4, and E_4, and they are synthesized mainly by leukocytes [2].

LTs are well known to promote leukocyte accumulation by increasing their generation in the bone marrow, their extravasation into tissues and survival at sites of inflammation. They also activate macrophages to release other proinflammatory mediators, such as TNF-α, IL-6, and IL-8, and growth factors such as fibroblast growth factor [3].

Here in this review we provide some studies involving the administration of LTB$_4$ in several animal and human models of infection and we explore the advantages of the microencapsulated LTB$_4$ as innovative pharmaceutical approach.

Since 2005, our group has constructed a research line by studying a biodegradable microparticulate system to deliver LTs, especially LTB$_4$, which could be tested in different *in vitro/in vivo* models. This technology brought to us the perspective to explore new strategies concerning the protection of lipid mediator molecule against oxidation and hydrolysis processes and also the sustained release of the LTs from microspheres to cell compartments at different models of infections.

Endogenous Production of LtB$_4$ and the Cellular Scenario in the Immunological Response

The potent chemoattractant LTB$_4$ binds, preferentially, to the high affinity LTB$_4$ receptor-1 (BLT1), which is present on polymorphonuclear neutrophils [4], eosinophils [3], effector T cells [5], peritoneal macrophages [6], dendritic cells [7], and mast cells [8]. The attachment of BLT1 by LTB$_4$ triggers a variety of cellular events *e.g.*: phosphoinositide-3 kinase and Akt, chemotaxis, intracellular Ca^{2+} mobilization, activation of extracellular signal-regulated kinase 1 and 2, degranulation, and/or the production of inflammatory proteins [2].

Despite evidences of general effects in proinflammatory responses, recent studies are trying to elucidate specific mechanisms by which LTB$_4$ drives effective host defense. Recently, authors have demonstrated that LTB$_4$/BLT1 ligation is indispensable for activation of Myeloid Differentiation Factor 88 (MyD88). This protein is responsible for signaling mediators through all of the known TLRs, except TLR3, and for producing IL-18, IL-1 β and NF- κB activation. The evidences suggest that LTB$_4$ signal transduction is indispensable for activation and/or increases Myd88 gene expression in macrophages and consequently produces responses against a variety of experimental infections [6]. In addition to its direct impact on TRLs signal transduction, LTB$_4$ also triggers the up-regulation of β2-integrins on monocyte surface [9].

Other evidence between LTB$_4$/BLT1 and innate immunity is about phagocytosis by macrophages. These cells act engulfing opsonized particles through the action of the receptors that recognize IgG Fc portion. Some authors have suggested the direct cross-talk between LTB$_4$/BLT1 and IgG-Fc on macrophage´s signaling, when they show that FcγRs-dependent phagocytosis was attenuated in BLT1-deficient macrophages as compared with wild-type cells. Moreover, LTB$_4$/BLT1 signaling restores phagocytosis in the absence of FcγRs signaling [10].

Regarding dendritic cells (DCs), LTB$_4$ produced from others cells is

important for NF- κB activation and starting IL-12 production through $LTB_4/BLT1$ signal transduction. LTB_4 produced by DCs is important as autocrine mechanism to cell migration. Consequently, the absence of BLT1 in experimental mice reduced DC stimulatory effects on the allogeneic naïve T cells to produce IFN-γ and TNF-α [7].

Innate immunity is dependent on neutrophils, which are responsible to synthesize LTs and to release antimicrobial peptides. The generation and function of this lysosomal enzyme are regulated by LTB_4 and, equally, the cathelicidin called LL-37 can affect the synthesis and function of this lipid mediator as a positive feedback loop that improves neutrophil responses in inflammatory processes [11].

With respect to lymphocyte responses, LTB_4 also influences T cells, because BLT1 is more expressed in effector T cells than those of the peripheral blood without inflammatory stimulus. Besides BLT1, effector T cells also express CCR7, suggesting that they can migrate to draining lymph nodes. Thus, BLT1+ T cells may interact with activated tissue resident or lymph node resident antigen-presenting cells to enable rapid local or systemic initiation and progression of the secondary immune response [12]. This fact credits the LTB_4 molecule as a potential link between innate and adaptive immunological reactions.

Finally, other authors obtained results suggesting the importance of LTB_4 on acquired immunity. Experiments with EBV-infected cord blood cell cultures showed that NK cells produce IFN-γ that acts on the monocytes, which produces LTB_4 and induce them to produce the cytokines IL-15, IL-12 and IL18. These interactions produce a positive feedback that will finally implicate in the selection of T cells [13].

In the context of host immune responses against pathogens, recent studies in animal models have shown that endogenous LTs display protective effect against a variety of infectious diseases, such as fungal pneumonia [14], infections caused by helminths [15] and bacterial peritonitis [16].

After the modulation of these cellular events, LTB_4 is inactivated through metabolic degradation by the microsomal ω-oxidation, mitochondrial and peroxisomal β-oxidation pathways. So, this molecule is not stored and released, but synthesized *de novo* from arachidonic acid in activated innate immune cells [17,18].

LtB_4 Binding with its Receptors and the Modulation of Inflammation

Three distinct receptors for LTB_4 have been identified [19-21]. The transmembrane receptor BLT1 is better described than the other two. It is characterized as a cell surface G protein coupled seven domain receptors and with a specific high affinity for LTB_4. This receptor is expressed predominantly on leukocytes including granulocytes, monocytes/macrophages, mast cells, dendritic cells and effector T cells [20]. Another specific receptors for LTB_4 are the so-called peroxisome proliferator-activated receptors (PPARs), which are situated in the cell nucleus. A sub-type receptor is PPAR-α, which is the nuclear receptor for eicosanoids including LTB_4 and their interaction promotes degradation of lipid mediators [19,21]. PPAR-α binds to PPAR responsive elements (PPREs) at the promoter sites of several lipid metabolism-related enzymes, such as LTB_4 ω-hydroxylases. LTB_4 binds and activates PPAR-α, resulting in the transcription of genes that promote fatty acid degradation. PPAR-α-deficient mice showed prolonged inflammatory responses in arachidonic acid-induced ear welling [19]. Moreover, PPAR-α can activate or inhibit arachidonic

acid-induced murine ear inflammation by enhancing the degradation of LTB_4 [22]. These data suggest that PPAR-α plays an important role in the clearance of lipid mediators during inflammation [18,21].

On the other hand, these transcription factors also regulate gene expression of enzymes associated with lipid homeostasis and affect the duration of an inflammatory response induced by LTB_4 [19]. Although the majority of studies have indicated an anti-inflammatory role of PPAR-α ligands, an increase in the neutrophil chemoattractant IL-8 and the MCP-1 levels have also been observed in endothelial cells [23]. In addition, the PPAR-α ligand fenofibrate was demonstrated to enhance nitric oxide synthase (NOS) expression and activity in isolated endothelial cells [24]. PPARs have been reported to regulate inflammatory responses, both *in vivo* and *in vitro*. In this context, PPAR-α activation by LTB_4 binding affects the duration of the inflammatory response induced by this eicosanoid [19]. Also, it is important to note that some studies report that PPAR-γ has no anti-inflammatory activity or might indeed exert a proinflammatory response [25].

Employment of LTB_4 Therapy to Treat Airway Infections: Immunological and Pharmaceutical Approaches

Some evidences demonstrated that LTB_4 is important to resolve infections and the administration of this compound during early infection is extremely efficient, but not as a preventive. The wild-type and 5-LO knockout mice were pretreated by administration of LTB_4 via the intraperitoneal, intravenous, and intranasal routes immediately before pneumococcal infection. After this, LTB_4 was administered by aerosol 24h later, in some animals. The pretreatment did not increase animal´s live, but the aerosolized LTB_4 was effective for improving lung bacterial clearance when administered postinoculation in animals with established infection [26]. These findings suggest the potential use of the lipid mediator as alternative strategy to control pulmonary infections but the understanding about the questions "when" and "how much" LTB_4 is necessary to confer effective protection seems to be the "big deal" involving these therapies.

Other authors examined dose- and time-dependent effects of nasal administration of LTB_4 in healthy subjects on symptoms. Accordingly, they monitored the neutrophil granule protein myeloperoxidase and α-defensins as index of neutrophil activity. Eosinophil cationic protein and α2- macroglobulin were monitored as eosinophil activity and plasma exudation, in order to explore whether or not LTB_4 had any consequence to the nasal mucosa. It was also studied whether or not exposure of neutrophils to LTB_4 *in vitro* induced virucidal effects against respiratory viruses: human coronavirus, respiratory syncytial virus and influenza B virus. Finally, in a preliminary experiment involving healthy subjects, they examined the effects of LTB_4 on human rhinovirus-16 induced replication, seroconversion, and symptoms [27]. The conclusion was that nasal administration of LTB_4 in humans produces increased nasal neutrophil activity, potentially reflecting an enhanced state of innate immune defense. Furthermore, LTB_4 presented a potential to exert antimicrobial effects on respiratory tract infections.

Recently, these studies involving the administration of LTs, especially LTB_4, in animal and human could bring consistent data about the features of lipid mediators in modulating innate and also adaptive immune responses against several pathogens. Indeed, these authors put a lot of their efforts in trying to investigate the variety

of roles elicited by LTs in the context of host defense mechanisms. However, pharmaceutical approaches besides immunological ones are essential to provide a real understanding about the behavior of immune cells when they are introduced to exogenous LTs, such as in a form of aerosolized solution or nasal instillation. It is worth to point out that lipid mediators are susceptible to oxidation and hydrolysis processes and the duration of the biological effect can be compromised.

In this context, technologies employing micro and nanoparticles for drug delivering purposes have obtained great impact in the fields of pharmacy and medicine. A great range of natural and synthetic polymers can be used to constitute different matrix types for encapsulating bioactive molecules. Particularly, particles made from poly lactic-co-glycolic acid (PLGA) polymer can be used as a delivery system and also provide adjuvant activity in immunization procedures [28,29]. These polymeric particulate delivery systems are able to present antigens and activate both humoral and cellular responses [29-31]. Many studies have discussed the ideal size of these particles in contributing to the generation of immune response [32-34]. Polymer-entrapped antigens reveal that micron-sized range particles promote humoral response whereas nanoparticles promote cellular response [35,36]. Regarding PLGA-based nanoparticles, they are extensively taken up by non-phagocytic eukaryotic cells, macrophages and dendritic cells [37]. Biodegradable micro/nanoparticles generated from PLGA have attracted attention due to their clinically proven biocompatibility [38]. In addition to facilitate the uptake of encapsulated materials, PLGA particles also potentially protect different molecules, such as nucleic acids, peptides and protein antigens, increasing delivery efficiency [39].

Microencapsulated LtB$_4$ in Biodegradable Polymer: A Pharmaceutical Contribution for Pushing Cells to Activation

With respect to the micro/nanotechnology, our group has published studies involving the development, characterization and biological application of microspheres containing LTB$_4$ (LTB$_4$-MS) for efficient drug delivering system [40,41]. The schematic representation of the manufacturing process for encapsulating LTB$_4$ in PLGA microspheres is shown in Figure 1.

In this context, after conducting some specific experiments, we could introduce new data in the literature about the use of PLGA microspheres containing lipid mediators, especially LTB$_4$, with the aim to modulate cell responses by increasing leukocyte recruitment in mouse model using intravital microscopy, phagocytosis process employing peritoneal macrophages and also cytokines' production in the lungs when those microspheres were intranasally administered to mice infected with the fungus *Histoplasma capsulatum*. With respect to the mechanisms of host defense stimulated by LTB$_4$ during infectious diseases, our group has studied the influence of this lipid mediator on antimicrobial responses against *H. capsulatum*. The published data [42] could bring good evidence showing that pulmonary administration of microspheres containing LTB$_4$ (LTB$_4$-MS) could inhibit fungal growth in the lungs of 5-LO$^{-/-}$ infected mice, suggesting the ability of these particles in restoring pulmonary LTB$_4$ levels by an exogenous way and also killing yeasts in the lungs. That proposed biodegradable microparticulate system, which could release LTB$_4$ to the lungs, was able to modulate inflammatory cytokine production related to host defense (Th1-mediated immune response), enhancing important killing mechanisms to inhibit fungal burden. With respect to histoplasmosis,
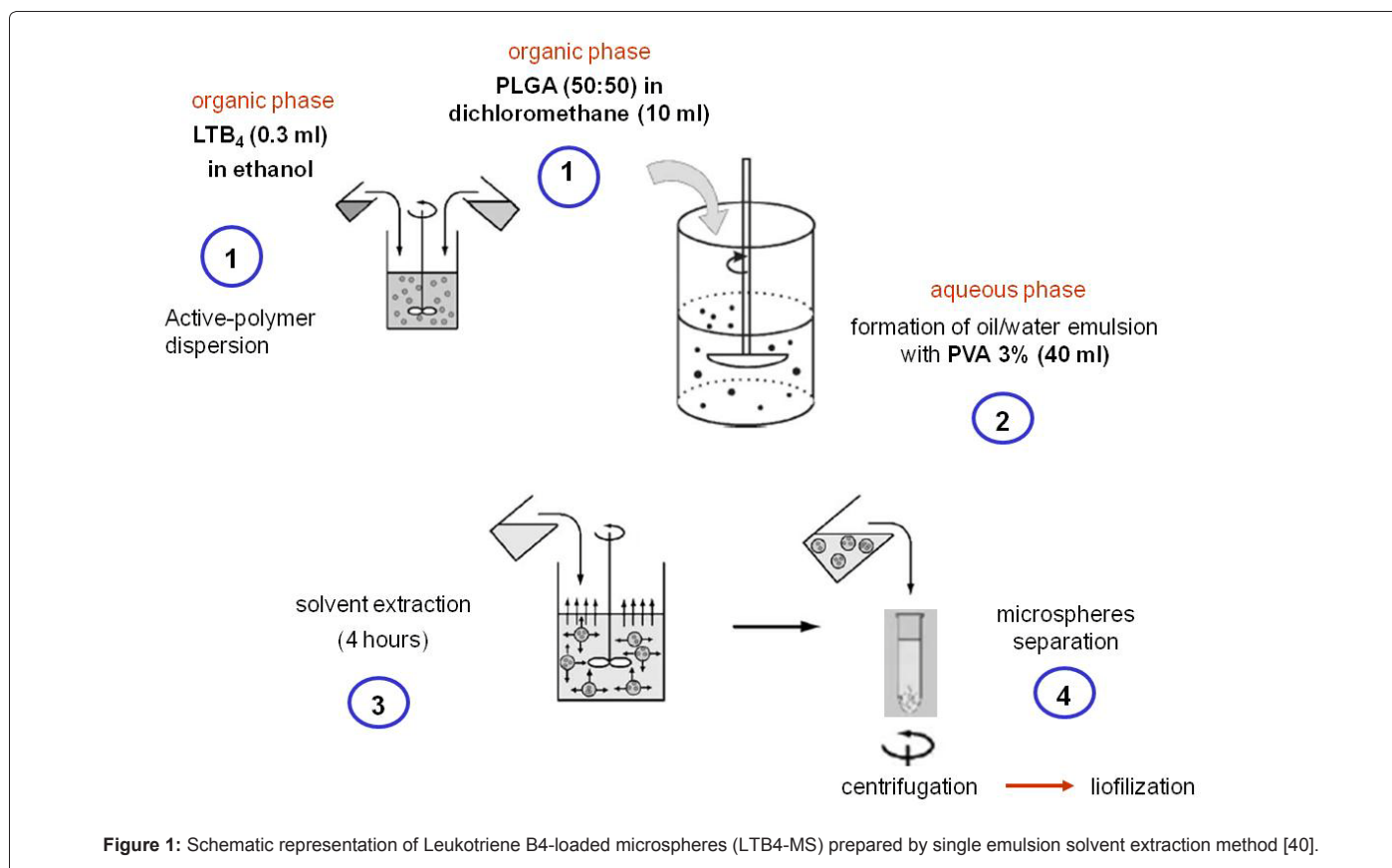

Figure 1: Schematic representation of Leukotriene B4-loaded microspheres (LTB4-MS) prepared by single emulsion solvent extraction method [40].

the fungal infection caused by *H. capsulatum*, our group has previously demonstrated that LTs are the main chemoattractants involved in the neutrophil, eosinophil and mononuclear cell migration induced by the fungus or cell wall fraction thereof [43].

Regarding this scenario involving cell activation after the administration of LTB_4-MS to mice, here we propose a schematic representation (Figure 2) showing the possible interactions of the microspheres with the LTs receptors, present in the cell membrane (BLTs) and nucleus (PPARs). Based on the illustration, we can speculate about the biological actions of LTB_4-MS either interacting with BLTs after the lipid mediator release from microspheres or being engulfed by phagocytic cells and further bound with PPARs inside the nucleus.

Regarding new attempts to enhance the antimicrobial activity of the host cells during murine histoplasmosis, we proposed the employment of a biodegradable microparticulate system, which could release LTB_4 to the lungs [42]. That approach, previously characterized, showed that PLGA microspheres containing LTB_4 were able to protect the lipid mediator against degradation [40] and also to confer a different type of cell activation when mouse and human cells were incubated with those microspheres [41].

It is also important to note that besides the potential application of these LTs delivery system against infectious diseases, on the other hand, the blockade of lipid mediators, like that employed for asthma therapy, is an effective treatment established. These two different therapy approaches can be perfectly fit with clinical observations concerning that asthmatic patients can develop predilection for pulmonary infections. This fact reveals an important modulating role for LTs in immune responses, especially in the context of LTB_4 released from the biodegradable microspheres to the lungs.

In order to get an illustration about the interference of classical chemotherapy on lipid mediators' production by the cells, the antifungal agent amphotericin B has been reported to inhibit neutrophil 5-LO metabolism [44] and this potentially undesirable action can be extended to other antimicrobials. Based on the fact that a relative state of LT deficiency characterizes many conditions associated with increased susceptibility to infection, the possibility that stimulation of innate and/or adaptive immunity might be accomplished by augmenting tissue levels of LTs merits consideration. In fact, it can be suggested that enhancing levels of LTs biosynthesis may indirectly contribute to the immunostimulation and effective host antimicrobial responses against pathogens.

Our *in vitro* studies using murine peritoneal macrophages showed that microencapsulated LTB_4 was able to induce greater nitric oxide production by those cells than LTB_4 in solution form, especially when the macrophages were preincubated with a specific BLT1 receptor antagonist, CP 105,696. Interestingly, when BLT1 receptors were blocked by this antagonist, an enhanced and significant response was observed [41]. This fact can be due to the different mechanisms of cell activation conferred by LTB_4 in solution form or released from MS. It is also likely that LTB_4 released from engulfed MS could exert its activity
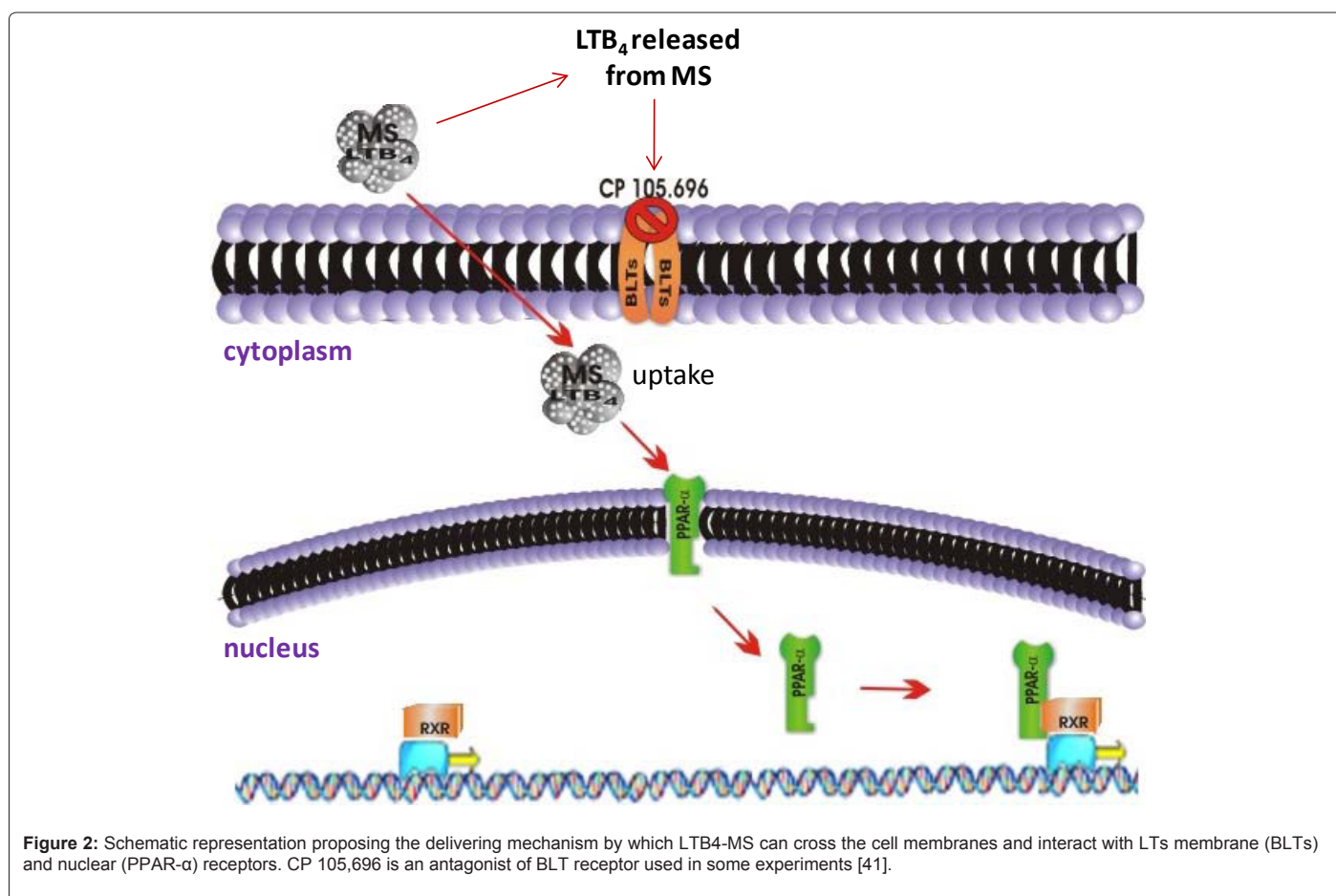
Figure 2: Schematic representation proposing the delivering mechanism by which LTB4-MS can cross the cell membranes and interact with LTs membrane (BLTs) and nuclear (PPAR-α) receptors. CP 105,696 is an antagonist of BLT receptor used in some experiments [41].

inside the cell nucleus, where it can bind to specific receptors, whereas exogenous LTB_4 in solution binds only to specific BLTs membrane receptors (Figure 2). As proposed in this figure, the LTB_4 binding to nuclear receptors without previous interaction with its membrane receptors (BLTs) results in an increase of PPAR-α expression and possibly activates the inflammatory genes to produce NO, MCP-1 and other inflammatory mediators [41].

During the last years, many studies have shown that LTs are involved in a range of infections by modulating the production of other inflammatory mediators [14,15], inducing phagocytosis [45] and activating antimicrobial mechanisms [46,47]. Regarding *H. capsulatum* infection, our group demonstrated that 14 days post-infection, the infected mice presented a marked decrease of LTB_4 production by the lung cells [14]. Moreover, when infected mice were treated with a 5-LO inhibitor (MK 886), it was observed 100% mortality and also lung CFU increase at 14 days post-infection. In this context, using LTB_4-MS as a delivery system, we could bring new approach to complement our efforts in elucidating the role of LTB_4 during host defense against histoplasmosis. In that study, we could observe that the pulmonary administration of LTB_4-MS provoked significant neutrophils migration to bronchoalveolar fluid in 5-LO$^{-/-}$ infected mice. This fact suggested that the lipid mediator had its biological activity preserved and could be released from microspheres during the course of infection, recruiting and activating important cells related to host defense against the fungus. With this regard, we previously demonstrated that LTB_4-MS were able to recruit high neutrophils numbers to bronchoalveolar space of 5-LO$^{-/-}$ mice [41]. In order to correlate those findings to fungal growth in the lungs, we could observe that LTB_4 released from microspheres could inhibit fungal growth, as reported by CFU recovery. Our results showed that LTB_4 is important to confer protection to animals, enhancing the antimicrobial responses of the infected cells, since 5-LO$^{-/-}$ mice had 2 $\log_{10}$ increase in lung CFU compared to sv129 mice [42].

Another point that we investigated during that study was the effect of microspheres´ administration on the inflammatory cytokines' production by the infected mice. It was demonstrated that IL-12, TNF-α and IFN-γ are involved in host defense against intracellular yeasts, such as *H. capsulatum* ones [48]. Our results showed that LTB_4-MS were able to induce the inflammatory cytokines' production by 5-LO$^{-/-}$ infected mice. This finding suggests the role of LTB_4 in modulating the T-helper 1 (Th1)-mediated immune response to *H. capsulatum*, a well established response against this infection [49]. Many studies have shown that LTB_4 is an important mediator for IL-2, IL-4, IL-12 and IFN- production by T-cells [49]. Also, other authors demonstrated that TNF-α blockade during the course of murine infection by *H. capsulatum* decreases protective immunity [50]. Taking together, these findings support the idea that LTB_4 released from microspheres to the lungs was able to modulate cytokines' production during critical days of the infection and also to recruit and activate important cells involved in antifungal responses.

In summary, our results concerning LTB_4 administration as therapy and more specifically, during *H. capsulatum* infection, provided evidence that the pulmonary administration of LTB_4-MS could increase leukocytes recruitment to bronchoalveolar space of 5-LO$^{-/-}$ infected mice. Also, the fungal growth in the lungs was significant inhibited by the microspheres administration. The proposed biodegradable microparticulate system, which can release LTB_4 to the lungs was able to modulate inflammatory cytokines' production related

to host defense, by enhancing important killing mechanisms to inhibit fungal burden. We believe that this approach can be employed to treat infectious diseases, besides histoplasmosis, as an alternative therapy or in association with other classical drugs.

Conclusions

As described in this review, great evidences support that LTs are important molecules in innate and also adaptive immune responses. Notable roles of these lipid mediators include their ability to be synthesized both rapidly and in delayed manner by a variety of cell types, their diverse antimicrobial actions, and their interactions with other relevant mediators. When compared with cytokines and chemokines, however, their role in antimicrobial defense has been largely underestimated. This likely reflects the common view that lipid mediators are exclusively pathogenic and their pharmacologic blockade has dominated the therapy established. However, new perspectives are needed to explore the potential therapeutic functions of lipid mediators, such as LTB_4, both in innate and adaptive immunities. In this context, as pointed out in this review, local LTB_4 administration has been shown to reduce the burden of bacteria, fungi and helminths. Also, this mediator has been administered to the human lung via aerosol [27,51] or via a bronchoscope [52] and resulted in neutrophil influx. On the other hand, it is know that LTB_4 is able to constrict isolated pulmonary arteries in guinea pig model [53] and also to promote human airway smooth muscle cells proliferation and migration [54]. Taking into consideration these biological activities of LTB_4, we can infer that the administration of this lipid mediator to the lungs can lead to side-effects, such as potent proinflammatory events, as described. So, the understanding about the precise time and amount of LTB_4 to be delivered to lungs' environment is critical for the establishment of holistic and more efficient therapies.

However, based on the evidence that LTB_4 molecule can be oxidized in solution form and several doses are required to maintain therapeutic levels during the treatment, as reported by the studies cited in this review, our research regarding the employment of biodegradable microspheres to release this mediator to infection sites, in a sustained manner, gains great importance. Since LTs can activate many antimicrobial mechanisms in the cells, our approach can constitute a different strategy to enhance host immune responses against a variety of pathogens.

References

1. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN (2001) Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol. 2: 612-619.

2. Ohnishi H, Miyahara N, Gelfand EW (2008) The role of Leukotriene B_4 in allergic diseases. Allergol Int 57: 291-298.

3. Faccioli LH, Nourshargh S, Moqbel R, Williams FM, Sehmi R, et al. (1991) The accumulation of 111In-eosinophils induced by inflammatory mediators, *in vivo*. Immunology 73: 222-227.

4. Canetti C, Silva JS, Ferreira SH, Cunha FQ (2001) Tumour necrosis factor-alpha and leukotriene B_4 mediate the neutrophil migration inimmune inflammation. Br J Pharmacol 134: 1619-1628.

5. Goodarzi K, Goodarzi M, Tager AM, Luster AD, von Andrian UH (2003) Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. Nat Immunol 4: 965-973.

6. Serezani CH, Lewis C, Jancar S, Peters-Golden M (2011) Leukotriene B_4 amplifies NF-κB activation in mouse macrophages by reducing SOCS1 inhibition of MyD88 expression. J Clin Invest 121: 671-682.

7. Toda A, Terawaki K, Yamazaki S, Saeki K, Shimizu T, et al. (2010) Attenuated Th1 induction by dendritic cells from mice deficient in the leukotriene B4 receptor 1. Biochimie 92: 682-691.

8. Metcalfe DD, Baram D, Mekori YA (1997) Mast cells. Physiol Rev 77: 1033-1079.

9. Vaddi K, Newton RC (1994) Regulation of monocyte integrin expression by β-family chemokines. J Immunol 153: 4721-4732.

10. Okamoto F, Saeki K, Sumimoto H, Yamasaki S, Yokomizo T (2010) Leukotriene B4 augments and restores Fc gammaRs-dependent phagocytosis in macrophages. J Biol Chem 285: 41113-41121.

11. Wan M, Sabirsh A, Wetterholm A, Agerberth B, Haeggström JZ (2007) Leukotriene B4 triggers release of the cathelicidin LL-37 from human neutrophils: novel lipid-peptide interactions in innate immune responses. FASEB J 21: 2897-2905.

12. Islam SA, Thomas SY, Hess C, Medoff BD, Means TK, et al. (2006) The leukotriene B4 lipid chemoattractant receptor BLT1 defines antigen-primed T cells in humans. Blood. 107: 444-453.

13. Klein E, Liu A, Claesson HE (2008) Activation of innate immunity by the leukotriene B 4 inhibits EBV induced B-cell transformation in cord-blood derived mononuclear cultures. Immunol Lett 116: 174-177.

14. Medeiros AI, Sá-Nunes A, Soares EG, Peres CM, Silva CL, et al. (2004) Blockade of endogenous leukotrienes exacerbates pulmonary histoplasmosis. Infect Immun 72: 1637-1644.

15. Machado ER, Ueta MT, Lourenço EV, Anibal FF, Sorgi CA, et al. (2005) Leukotrienes play a role in the control of parasite burden in murine strongyloidiasis. J Immunol 175: 3892-3899.

16. Malaviya R, Abraham SN (2000) Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. J Leukoc Biol 67: 841-846.

17. Murphy RC, Gijón MA (2007) Biosynthesis and metabolism of leukotrienes. Biochem J 405: 379-395.

18. Yokomizo T, Izumi T, Shimizu T (2001) Leukotriene B_4: Metabolism and signal transduction. Arch Biochem Biophys 385: 231-241.

19. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, et al. (1996) The PPAR alpha-leukotriene B4 pathway to inflammation control. Nature 384: 39-43.

20. Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T (1997) A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. Nature 387: 620-624.

21. Narala VR, Adapala RK, Suresh MV, Brock TG, Peters-Golden M, et al. (2010) Leukotriene B4 is a physiologically relevant endogenous peroxisome proliferator-activated receptor-α agonist. J Biol Chem 285: 22067-22074.

22. Colville-Nash P, Willis D, Papworth J, Freemantle C, Lam C, et al. (2005) The peroxisome proliferator activated receptor alpha activator, Wy14,643, is anti-inflammatory in vivo. Inflammopharmacology. 12: 493-504.

23. Lee H, Shi W, Tontonoz P, Wang S, Subbanagounder G, et al. (2000) Role for peroxisome proliferator-activated receptor alpha in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells. Circ Res. 87: 516-521.

24. Goya K, Sumitani S, Xu X, Kitamura T, Yamamoto H, et al. (2004) Peroxisome proliferator-activated receptor alpha agonists increase nitric oxide synthase expression in vascular endothelial cells. Arterioscler Thromb Vasc Biol 24: 658-663.

25. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM (1998) PPAR gamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 93: 241-252.

26. Mancuso P, Lewis C, Serezani CH, Goel D, Peters-Golden M (2010) Intrapulmonary administration of leukotriene B4 enhances pulmonary host defense against pneumococcal pneumonia. Infect Immun 78: 2264-2271.

27. Widegren H, Andersson M, Borgeat P, Flamand L, Johnston S, et al. (2011) LTB4 increases nasal neutrophil activity and conditions neutrophils to exert antiviral effects. Respir Med 105: 997-1006.

28. Jiang W, Gupta RK, Deshpande MC, Schwendeman SP (2005) Biodegradable poly (lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. Adv Drug Deliv Rev 57: 391-410.

29. O'Hagan DT, Singh M (2003) Microparticles as vaccine adjuvants and delivery systems. Expert Rev Vaccines 2: 269-283.

30. Men Y, Audran R, Thomasin C, Eberl G, Demotz S, et al. (1999) MHC class I- and class II-restricted processing and presentation of microencapsulated antigens. Vaccine 17: 1047-1056.

31. Thiele L, Rothen-Rutishauser B, Jilek S, Wunderli-Allenspach H, Merkle HP, et al. (2001) Evaluation of particle uptake in human blood monocyte-derived cells in vitro. Does phagocytosis activity of dendritic cells measure up with macrophages? J Control Release 76: 59-71.

32. Carcaboso AM, Hernández RM, Igartua M, Rosas JE, Patarroyo ME, et al. (2004) Potent, long lasting systemic antibody levels and mixed Th1/Th2 immune response after nasal immunization with malaria antigen loaded PLGA microparticles. Vaccine 22: 1423-1432.

33. Tabata Y, Ikada Y (1988) Macrophage phagocytosis of biodegradable microspheres composed of L-lactic acid/glycolic acid homo- and copolymers. J Biomed Mater Res 22: 837-858.

34. Eldridge JH, Staas JK, Meulbroek JA, McGhee JR, Tice TR, et al. (1991) Biodegradable microspheres as a vaccine delivery system. Mol Immunol 28: 287-294.

35. Gutierro I, Hernández RM, Igartua M, Gascón AR, Pedraz JL (2002) Size dependent immune response after subcutaneous, oral and intranasal administration of BSA loaded nanospheres. Vaccine 21: 67-77.

36. Chong CS, Cao M, Wong WW, Fischer KP, Addison WR, et al. (2005) Enhancement of T helper type 1 immune responses against hepatitis B virus core antigen by PLGA nanoparticle vaccine delivery. J Control Release 102: 85-99.

37. Lutsiak ME, Robinson DR, Coester C, Kwon GS, Samuel J (2002) Analysis of poly (D,L-lactic-co-glycolic acid) nanosphere uptake by human dendritic cells and macrophages in vitro. Pharm Res 19: 1480-1487.

38. Bazile DV, Ropert C, Huve P, Verrecchia T, Marlard M, et al. (1992) Body distribution of fully biodegradable 14C-poly (lactic acid) nanoparticles coated with albumin after parenteral administration to rats. Biomaterials 13: 1093-1102.

39. Panyam J, Labhasetwar V (2002) Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Deliv Rev 55: 329-347.

40. Nicolete R, Lima Kde M, Júnior JM, Baruffi MD, de Medeiros AI, et al. (2007) In vitro and in vivo activities of leukotriene B_4-loaded biodegradable microspheres. Prostaglandins Other Lipid Mediat 83: 121-129.

41. Nicolete R, Rius C, Piqueras L, Jose PJ, Sorgi CA, et al. (2008) Leukotriene B4-loaded microspheres: a new therapeutic strategy to modulate cell activation. BMC Immunol 9: 36.

42. Nicolete R, Secatto A, Pereira PA, Soares EG, Faccioli LH (2009) Leukotriene B4-loaded microspheres as a new approach to enhance antimicrobial responses in Histoplasma capsulatum-infected mice. Int J Antimicrob Agents 34: 365-369.

43. Medeiros AI, Silva CL, Malheiro A, Maffei CM, Faccioli LH (1999) Leukotrienes are involved in leukocyte recruitment induced by live Histoplasma capsulatum or by the β-glucan present in their cell wall. Br J Pharmacol 128: 1529–1537.

44. Shindo K, Fukumura M, Ito A (1998) Inhibitory effect of amphotericin B on leukotriene B4 synthesis in human neutrophils in vitro. Prostaglandins Leukot Essent Fatty Acids 58: 105-109.

45. Mancuso P, Nana-Sinkam P, Peters-Golden M (2001) Leukotriene B_4 augments neutrophil phagocytosis of Klebsiella pneumoniae. Infect Immun 69: 2011-2016.

46. Serezani CH, Aronoff DM, Jancar S, Mancuso P, Peters-Golden M (2005) Leukotrienes enhance the bactericidal activity of alveolar macrophages against Klebsiella pneumoniae through the activation of NADPH oxidase. Blood 106: 1067-1075.

47. Peres CM, Aronoff DM, Serezani CH, Flamand N, Faccioli LH, et al. (2007) Specific leukotriene receptors couple to distinct g proteins to effect stimulation of alveolar macrophage host defense functions. J Immunol 179: 5454-5461.

48. Allendoerfer R, Biovin GP, Deepe GS Jr (1997) Modulation of immune responses in murine pulmonary histoplasmosis. J Infect Dis 175: 905-914.

49. Morita H, Takeda K, Yagita H, Okumura K (1999) Immunosuppressive effect of leukotriene B(4) receptor antagonist in vitro. Biochem Biophys Res Commun 264: 321-326.

50. Deepe GS Jr, Gibbons RS (2008) TNF-α Antagonism Generates a Population of Antigen-Specific CD4+CD25+ T Cells That Inhibit Protective Immunity in Murine Histoplasmosis. J Immunol 180: 1088-1097.

51. Sampson SE, Costello JF, Sampson AP (1997) The effect of inhaled leukotriene B4 in normal and in asthmatic subjects. Am J Respir Crit Care Med 155: 1789-1792.

52. Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA (1989) Effect of leukotriene B4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. J Clin Invest 84: 1609-1619.

53. Sakata K, Dahlén SE, Bäck M (2004) The contractile action of leukotriene B4 in the guinea-pig lung involves a vascular component. Br J Pharmacol 141: 449-456.

54. Watanabe S, Yamasaki A, Hashimoto K, Shigeoka Y, Chikumi H, et al. (2009) Expression of functional leukotriene B4 receptors on human airway smooth muscle cells. J Allergy Clin Immunol 124: 59-65.