

Leukotriene B4 enhances innate immune defense against the puerperal sepsis agent Streptococcus pyogenes

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Background

Worldwide, more than 500,000 women die per year as a complication of childbirth [1]. Infection is second only to hemorrhage as the major cause of maternal mortality. Streptococcus pyogenes (Streptococcus Group A or GAS) is a leading cause of severe postpartum sepsis,

globally responsible for > 75,000 maternal deaths per year [2]. Thus, there is a need for more research into the root causes of puerperal sepsis. We know that lipid mediators are important against infections and we focus on leukotriene B4 (LTB4), which is synthesized from arachidonic acid when an acute Ca²⁺ flux is induced in cells following activation by inflammatory mediators or pathogens [3, 4]. LTB4 has been shown to augment macrophage immune defenses against several pathogens both in vitro and in vivo. However, little is known about whether LTB4 is present in the gravid uterus or regulates innate immunity in that setting. Signaling by LTB4 –BLT1 coupling regulates macrophage behaviors by reducing cAMP production, but this has not been studied in reproductive tract macrophages. Understanding this could unlock new pharmacological approaches to treating or preventing sepsis in new mothers.

Introduction

Postpartum sepsis is a major cause of the more than 500,000 maternal deaths that occur every year, and group A Streptococcus (GAS; *S. pyogenes*) is one of the leading etiologic agents. Gaps in our knowledge of the risk factors for postpartum GAS sepsis present major obstacles towards reducing this threat. The incidence of invasive GAS is more than 25 times higher for postpartum vs. nonpregnant women [5]. GAS infections range from mild endometritis to highly-lethal necrotizing infections with toxic shock [6, 7]. Puerperal GAS is an important cause of maternal death in resource-poor settings [8], and it is a reemerging problem in industrialized countries.

Leukotriene B4 is synthesized from arachidonic acid when an acute Ca²⁺ flux is induced in cells following activation by inflammatory mediators or pathogens [4]. Two G protein coupled receptors, BLT1 (high affinity) and BLT2 (low affinity), have been identified as mediating the actions of LTB4 [9]. LTB4 has been shown to augment macrophage immune defenses against several pathogens both in vitro and in vivo. However, little is known about whether LTB4 is present in the gravid uterus or regulates innate immunity in that setting.

The capacity of LTB4 to regulate host-streptococcal interactions has been shown in a model of lung infection caused by *S. pneumoniae* [10]. In addition, the administration of aerosolized LTB4 to mice with pre-existing pneumococcal pneumonia increased mononuclear phagocyte/macrophage accumulation in the lungs, NADPH oxidase subunit expression in pulmonary macrophages, and pulmonary bacterial clearance. While these data provide an important rationale for our hypothesis, nothing is known about the role of LTB4 in GAS infections. Accordingly, there is a need of additional studies with LTB4 to address the relation between LTB4 and GAS.

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Materials and Methods

The intrauterine infection, phagocytosis, killing assays and inflammatory mediators measures were performed according to the literature (adapted from [11-15]). Depending on the experiment we used: THP-1 cells, Peripheral Blood Mononuclear Cells, Placental (PMs), Decidual Macrophages (DMs) and mouse peritoneal macrophages for in vitro experiments. We also used C57BL/6 wild type (WT) or 5 lipoxygenase deficient mice (5LO^{-/-}) mice on a C57BL/6 background for in vivo experiments. Cell cultures for placental and decidual macrophages were performed and adapted according Thelen, et al [11]. Also we evaluated whether LTB₄ induces changes in cAMP or Ca²⁺ and whether these changes mediate actions of LTB₄ on antimicrobial functions. THP-1 cells were used to analyze the production of Ca²⁺ and cAMP. Cells were culture overnight in 6-well plates in RPMI1640 plus 10% FBS at a concentration of 3 x 10⁶

cells/well. Medium was changed to serum free medium and cells incubated for 15 min with or without LTB₄ [16]. Culture supernatants were aspirated and the cells lysates used to measure intracellular cAMP levels by ELISA according to the manufacturer (Enzo Life Sciences). For Ca²⁺ mobilization analysis, 4 x 10⁵ cells were cultivated in RPMI 1640 in 96-well plates specific for fluorescence for 1 hr and the media was removed and the dye solution (Fluo-4NW, Molecular Probes) added in the wells for 45 min at 37 °C, followed by addition of A23187 1µM (positive control; Cayman Chemical) or LTB₄ (10 nM) and subjected to intracellular fluorescence measurements per the manufacturer instructions.

Results and Discussion

We know that leukotrienes, including LTB₄, are important endogenous regulators of immune defense against bacterial pathogens [10, 17-19], suggesting that they might be involved in supporting host defense against GAS. We saw that 5LO^{-/-} mice were significantly more susceptible to overwhelming infection caused by GAS than WT mice and intrauterine infection with GAS significantly increased LTB₄ production in the WT uterus. An interestingly increase of IL-1, IL-6, IL-17 and MCP-1 in the uterine tissues of infected 5LO^{-/-} mice was observed when compared with WT animals. After that we started with in vitro experiments, where LTB₄ significantly increased the phagocytosis of unopsonized GAS by THP-1 cells, human peripheral blood monocytes, human DMs, human PMs, and mouse peritoneal macrophages. We used THP-1 cells to build our model since we saw the same effect with all macrophages. We confirmed that the mechanism by which LTB₄ can participate in the host defense against GAS is due the G_i activation and BLT1 receptor dependent. This mechanism is also dependent on the decrease of cAMP levels, but Ca²⁺ independent. Another important mechanism along with phagocytosis, it

is the microbicidal activity which is an important step in the control of infection. Previous work in other model systems has revealed that PGE₂ can suppress the capacity of macrophages to kill bacteria [20-22] while endogenous and exogenous leukotrienes enhances this action. So, we observed that the number of intracellular bacteria present in THP-1 cells following 30 min of incubation with GAS was significantly reduced by treating cells with LTB₄ (10 nM). Knowing that reactive oxygen intermediates are important for GAS clearance we tested the NADPHox activity using the selective NADPHox inhibitor apocynin. LTB₄ failed to reduce the number of intracellular bacteria in macrophages pretreated with apocynin, suggesting that LTB₄'s actions depend upon NADPHox activation.



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Conclusions

These investigations will significantly advance our understanding of the mechanisms that can contribute to GAS infections in human female reproductive tract. The data from this study may be contributed to understanding the mechanisms, as well as, for the discovery of therapeutic targets to help against infections. In summary the ligation of BLT1 by LTB4 results in the activation of G i protein, with a subsequent decrease in intracellular cAMP levels. This, in turn, limits the activation of protein kinase A (PKA), an endogenous suppressor of both phagocytosis and NADPHox-dependent reactive oxygen intermediate (ROI) generation.

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