The Role of Leukotriene B_4 Receptor BLT1 in Experimental Lyme Arthritis

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Introduction

- Leukotriene B_4 (LTB_4) is a potent lipid chemoattractant derived from arachidonic acid **(Figure 1)**. It is produced and released by neutrophils, mast cells, and macrophages during the innate immune response to infection.
- LTB_4 binds to its high-affinity receptor BLT1, located predominantly on the surface of leukocytes.
- Upon binding, LTB_4 induces neutrophil and monocyte chemotaxis to the site of inflammation.
- LTB_4 and BLT1 act in concert to produce tissue inflammation during autoimmune arthritis.
- Previous studies in autoimmune arthritis have shown that inhibition of BLT1 prevents recruitment of neutrophils and macrophages.
- We hypothesized that C3H BLT1$^{-/-}$ mice infected with *Borrelia burgdorferi* would exhibit less inflammation than C3H wild-type (WT) mice, as well as a decreased ability to clear *Borrelia*.

Simplified Eicosanoid Production Pathway

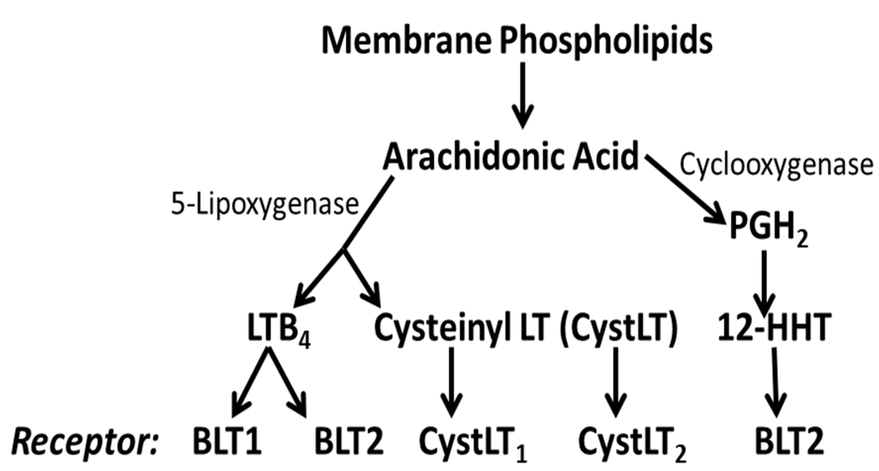

Figure 1: Simplified schematic of eicosanoid production, emphasizing leukotriene synthesis and their receptors.

LTB_4 Function in Inflammation

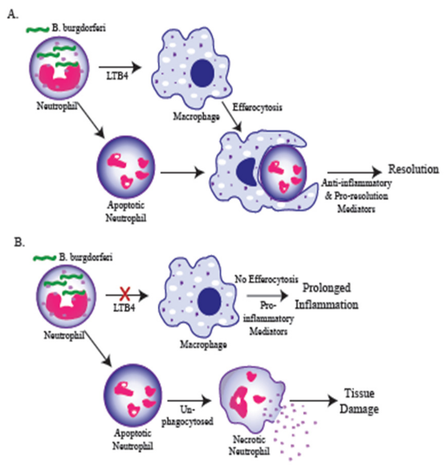

Figure 2: (A) LTB_4 enhances macrophage phagocytosis. **(B)** Lack of LTB_4 delays resolution.

Ankle Swelling Curve

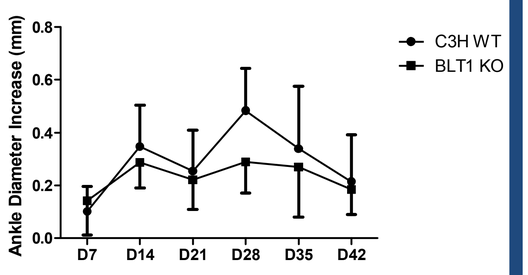

Figure 3: BLT1$^{-/-}$ mice exhibited similar swelling as C3H WT mice on consecutive days post-infection.

Materials and Methods

Infection: Stocks of virulent, N40 strain of *Borrelia burgdorferi* were grown to log phase in BSK medium with 6% rabbit serum. Spirochetes were counted and diluted in sterile BSK medium to 1x10^6 bacteria/ml. WT C3H/HeJ and C3H BLT$^{-/-}$ mice were inoculated in both hind footpads with 1x10^5 bacteria. Five mice per group were sacrificed 21 and 42 days post-infection. ***In Vivo* Cell Isolation/Flow Cytometry:** The right ankle was harvested from each mouse and placed into 5mL 1xPBS + 4% FBS containing DNaseI and collagenase dispase. The ankle was shredded, filtered through a 70 um filter, and washed in 1x PBS + 2% FBS prior to counting. Cells were stained with the following antibodies: F4/80, B220, IgM, Ly6g, CD45.2 and CD3e. Data was collected on a DAKO Cyan and analyzed with Summit ver 5.0 software.
Extraction and Measurement of LTB_4 and Lipoxin A_4: Ankle tissue samples were harvested and snap-frozen in liquid nitrogen at time of collection. LTB_4 and Lipoxin A_4 levels were measured using an LTB_4 Enzyme Immunoassay Kit from Cayman Chemical, and a Lipoxin A_4 Enzyme Immunoassay Kit from Oxford Biomedical Research, respectively.
Determination of Tissue *Borrelia* Loads: DNA was extracted from ankle joints by homogenization in TRIzol reagent according to manufacturer's protocol. Multiplex real-time (qRT) PCR was performed and normalized to copies of mouse *nidogen* DNA within the same sample.
Assessment of Arthritis Pathology: Progression of arthritis development was monitored by measuring ankle swelling through the thickest craniocaudal portion of the ankle joints using a metric caliper. Joint thickness was determined prior to infection and then at indicated time points throughout the course of infection. Ankle swelling measurements were then determined by subtracting the initial baseline measurement from subsequent measurements.

Ankle Histology

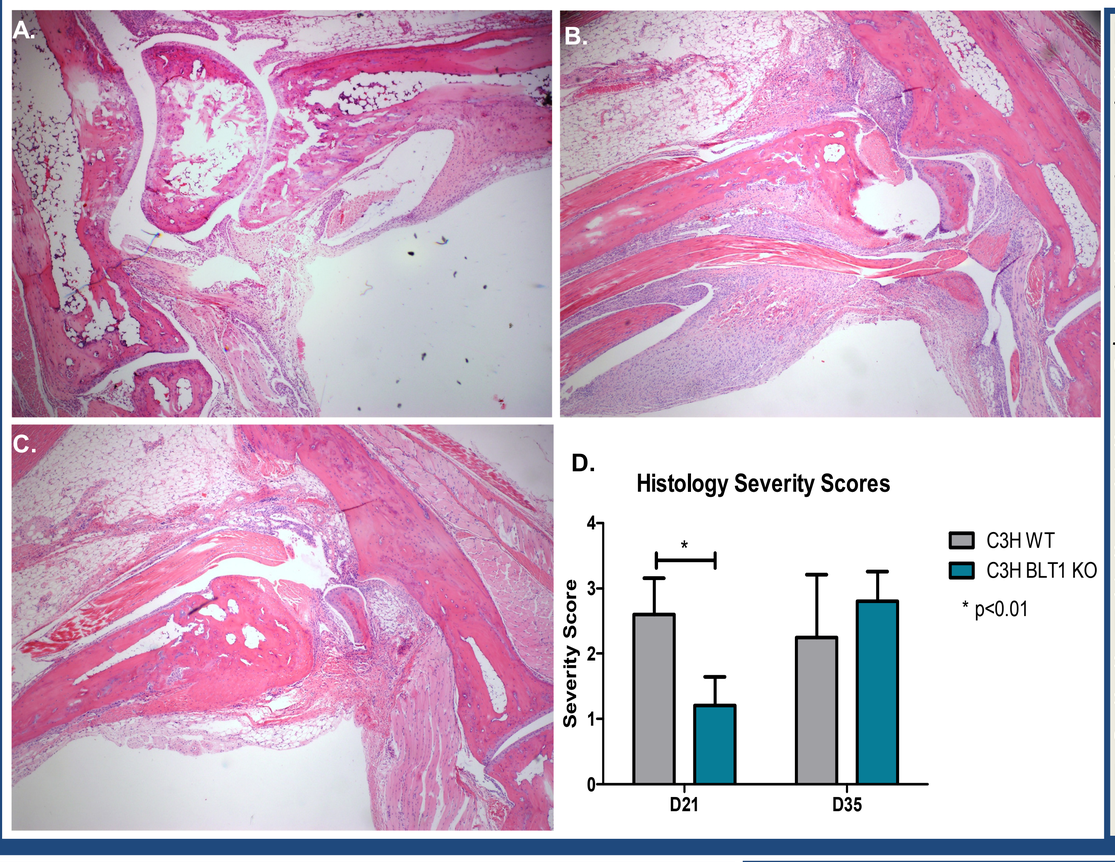

Figure 4: Arthritis severity, ranked from a score of 0 indicating normal tissue to 4. **(A-C)** H&E histology sections of left ankle tissue. **(A)** Normal joint, severity score 0. **(B)** WT C3H infected mouse, severity score 2, day 21. **(C)** C3H BLT1$^{-/-}$ infected mouse, severity score 3, day 21. **(D)** BLT1$^{-/-}$ mice had statistically significant less severe inflammation compared to WT mice on day 21.

Ear Tissue *Borrelia* Loads

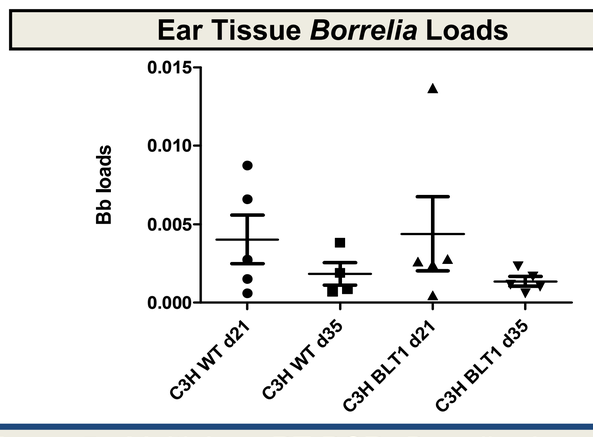

Figure 5: Multiplex qRT-PCR. *Borrelia* clearance from ear tissue was similar between mouse strains.

Eicosanoid Levels in Harvested Ankle Tissue

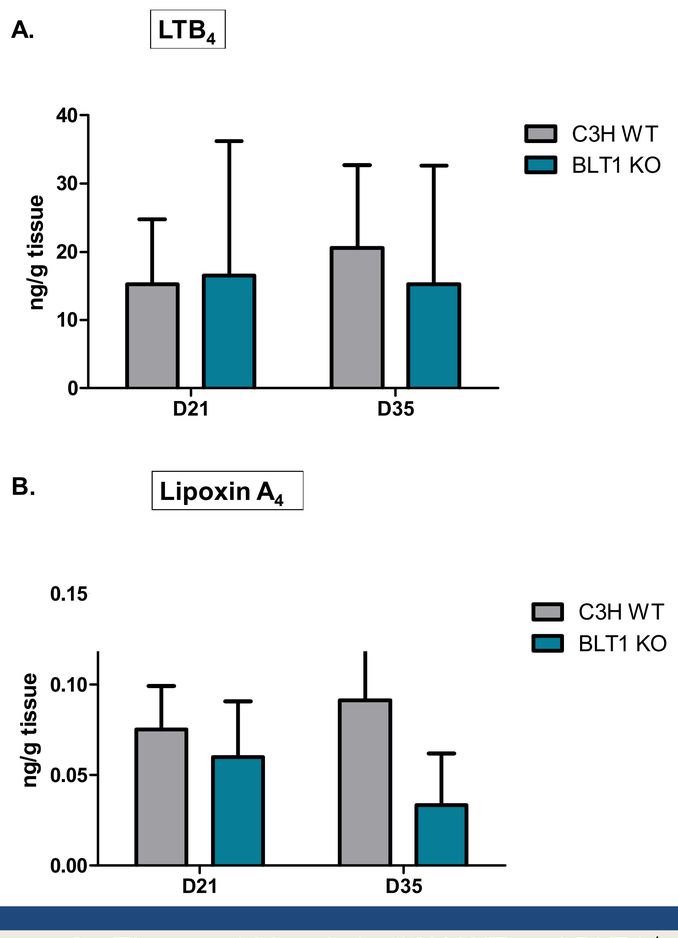

Figure 6: Eicosanoid levels in C3H WT and BLT1$^{-/-}$ mice, measured on days 21 and 35 post-infection. **(A)** LTB_4 levels were not significantly different between mouse strains. **(B)** LipoxinA_4, an anti-inflammatory eicosanoid, appeared to be present in decreased amounts in BLT$^{-/-}$ mice on day 35, but were not statistically significant.

Results

Arthritis severity scores demonstrate that the development of Lyme arthritis at the time of peak inflammation on day 21 appeared delayed in BLT1$^{-/-}$ as compared to C3H WT mice. However, 35 days post-infection, the arthritis severity scores of BLT1$^{-/-}$ mice were higher than WT mice, though not statistically significant. *Borrelia* ear loads were similar between both strains of mice. Flow cytometry data revealed similar cell populations between both mouse strains.

Flow Cytometry Numbers

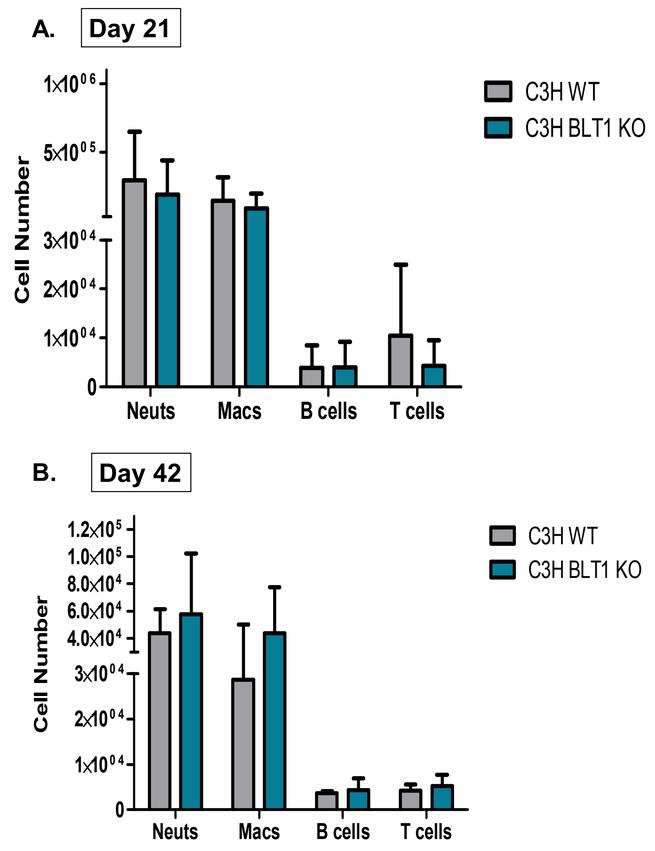

Figure 7: Cell counts between BLT1$^{-/-}$ and C3H WT mice as measured on days 21 and 42 were not statistically different.

Conclusions

1. *Borrelia* loads were not statistically different between the two strains, suggesting that another mechanism is at work which is clearing the pathogen in the absence of BLT1-mediated neutrophil and macrophage recruitment.
2. We expected LTB_4 levels to be increased in BLT1$^{-/-}$ mice, as the eicosanoid would be unable to bind to its receptor, but was not statistically different between the two strains of mice.
3. There appears to be a delay in development of arthritis in BLT1$^{-/-}$ mice.

Future Directions

1. Repeat experiment to confirm current findings.
2. Submit ankle tissue samples to more accurately measure levels of LTB_4, LipoxinA_4, and other eicosanoids using MS/MS analysis.
3. Examine tissue samples on day 60 post-infection to definitively conclude whether there is a delay in resolution of arthritis.
4. Measure levels of inflammatory and anti-inflammatory cytokines such as IL-12, IL-10, IL-6, IL-1β, and TNF-α.
5. Repeat experiment evaluating BLT2, the low-affinity LTB_4 receptor, in C3H BLT2$^{-/-}$ or inhibited mice.