Innate Immune Response in Candida albicans Endophthalmitis: the Role of Toll-Like Receptor Signaling
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INTRODUCTION

The term ‘endophthalmitis’ refers to intraocular inflammation that arises as a result of microbial infection. A wide range of pathogens, including both bacteria and fungi, are known to cause endophthalmitis. These microbes can be introduced to the eye from outside the body (traumatic) or inside the body (vasculature) of the blood.

While there have been many studies on the pathogenesis of bacterial endophthalmitis, very few studies have focused on fungal endophthalmitis. As a result, our understanding of this disease is limited. While fungal endophthalmitis is less common than bacterial endophthalmitis, it still presents a serious challenge in humid climates, rural areas, and in developing countries with unsupervised care. Cases of endophthalmitis from fungi cause increase each year.

In this study, we sought to improve our model of fungal endophthalmitis using Candida albicans (SC5314) as our model organism. We induced endophthalmitis via intraocular injection and observed the pathogenesis of the disease and the progression of the inflammatory response by various means. Upon standardizing our protocol in Wild Type C57BL/6 mice, we performed preliminary experiments in TLR KO mice to determine the importance of Toll-like receptor (TLR) signaling in the innate immune response and the pathogenesis of this disease.

METHODS

1. Developing a mouse model of fungal endophthalmitis

2. Candida albicans Induces Extensive Retinal Tissue Damage in Endophthalmitis

3. Pro-Inflammatory Cytokines are Produced Following Infection with Candida albicans

4. Infection with Candida albicans Leads to Neutrophil Infiltration in the Retina

5. Candida albicans - Infected Eyes Exhibit Rapid Decline in Retinal Function

RESULTS

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CONCLUSIONS

We were able to establish a mouse model of fungal endophthalmitis in Wild Type C57BL/6 mice.

The pathogenesis of the disease and the progression of the inflammation were observed via various means and analyzed extensively to understand the mechanisms of pathogenesis.

With the advancement of this mouse model of fungal endophthalmitis, many future studies can now be performed that will allow us to elucidate the mechanism of response and immunity to the disease.

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Figure 1: Endophthalmitis induced by WT C53858 mice via intra-retinal injection of 4x10^4 CFU of Candida albicans (strain SC5314).

Figure 2: Following injection with Candida albicans, disease progression was monitored daily via slit lamp photography. The mice were anaesthetized in the indicated time points and a slit lamp was used to take photographs of each eye (A: representative image) (10x, n=4 for each time point).

Figure 3: The levels of pro-inflammatory cytokines were measured in whole-eye lysates (ELISA) or retinal tissue lysates (qPCR).

Figure 4: Representative images of the retinas of WT and TLR4 KO C57BL/6 mice were harvested at 72 hours post-infection. For qPCR, manual retinal tissue was isolated and subjected to lysis. For ELISA, whole eye lysates were made by crushing with stainless steel beads. The levels were then subject to collection and the supernatant used for ELISA. Protein quantitation was used to calibrate the amount of protein in each well (µg/µL). The trend is similar for all these cytokines, with an increase in production for 0-24 hours, followed by a peak at 48 hours, and a decrease in production toward normal by 72 hours. For each qPCR and ELISA, statistical analysis was performed via two-way ANOVA for treatment by time interaction, followed by Sidak’s multiple comparison test.

Figure 5: ELISA analysis of WT and TLR4 KO mice shows a significant difference in IL-6 and TNF-α levels. While WT and IL-1β KO (C57BL/6) is greater in the WT mice at each time point, with both qPCR-PCR and ELISA show to be significant ‘p<0.001’, ‘p<0.005’, ‘p<0.0005’, ‘p<0.00005’, ‘p<0.000005’, ‘p<0.0000005’, respectively (two-way ANOVA).

Figure 6: qPCR was performed on manual retinal tissue from WT and C57BL/6 mice at the indicated time points post-infection. Significant expression was seen in TLR 1-4, 6, and 8/18, indicating that these TLRs play a role in fungal endophthalmitis. It is well known that TLR2 is important in the recognition of numerous pathogens, including both bacterial and fungal, and interacts with either TLR 1 or TLR 6 on the surface of immune cells. Given its importance in the immune response, we choose to investigate the significance of this pathway in our models. We performed a parallel experiment using WT and TLR2 KO C57BL/6 mice. Endophthalmitis was induced via intraocular injection of C. albicans and disease progression was monitored by slit lamp photography and fundus photography. Representative images.

There is no significant difference in TNF-α and IL-1β levels between WT and TLR2 KO mice. However, TLR2 KO mice were significantly different from WT mice at 72 hours post-infection, with IL-1β KO mice showing significant levels of IL-1β.

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