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VEGF, VEGF-C, and VEGF-D Increases in Aqueous Humor Samples in a LPS Induced Model of Uveitis in New Zealand White Rabbits

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Abstract

Purpose- To determine the levels of VEGF, VEGF-C, and VEGF-D from aqueous humor samples in an *E.coli* lipopolysaccharide (LPS) induced model of uveitis in rabbits.

Methods- Uveitis was induced by intravitreal (IVT) injection of LPS into the right eye(OD) of New Zealand White (NZW) rabbits. The left eye (OS) received an IVT injection of vehicle. At 24 hours post injection rabbits were evaluated by an ophthalmic exam and the aqueous humor (AH) collected for cell counts and protein. VEGF, VEGF-C, and VEGF-D were quantitated in AH using a Meso Scale Discovery (MSD) electrochemiluminescent ELISA.

Results- Ophthalmic exams of LPS treated rabbit eyes (OD) indicated substantial inflammation compared to control eyes (OS). Cell counts from OS eyes were very low or undetectable and increased to 2.1 x 10^6 cells/ml of AH in OD eyes. Protein from OS eyes averaged 3.2 mg/ml and increased to 32.9 mg/ml in OD eyes. VEGF and VEGF-C were below detection limits in OS eyes and increased to 7.1 pg/ml and 75.5 pg/ml, respectively, in OD eyes. VEGF-D in OS eyes averaged 96.0 pg/ml and increased to 853 pg/ml in OD eyes.

Conclusions- VEGF, VEGF-C, and VEGF-D levels increased significantly in LPS treated eyes of NZW and are indicative of an inflammatory response. Ophthalmic exams, cell counts, and protein increases in LPS treated eyes support increased inflammation in this rabbit model of uveitis. The significantly increased levels of VEGF-C and VEGF-D in AH are new findings.

Introduction

Uveitis induced by intravitreal injection of lipopolysaccaride (LPS) is a widely used animal model of clinical uveitis. Following IVT of LPS a characteristic infiltration of leukocytes and protein leakage into the eye is observed. Various factors and cytokines such as C5a, LTB4, platelet activating factor (De Vos and Kijlsra, 1992; Herbort, Okumura and Mochzuli, 1998; Lin et al., 1991; Mondino and Sumner, 1986) and TNFα and IL-1β (Jun-Song Mo et al, 1998) have been implicated as mediators in the development and pathogenesis of uveitis. In this study we tested a commercially available human Angiogenesis Panel from (MSD) in a rabbit LPS induced uveitis model for its ability to detect increased levels of protein associated with angiogenesis in rabbit aqueous humor. A distinct advantage of using the MSD Angiogenesis Panel is multiplexing, or the ability to test for changes in the levels of multiple proteins from a small volume of aqueous humor.

Materials and methods

LPS Administration

NZW rabbits were anesthetized via isoflurane inhalation prior to intravitreal (IVT) injections. At least 2 minutes prior to dosing, the eye to be dosed was moistened with an ophthalmic Betadine solution. After approximately 2 minutes, the Betadine was washed out of the eyes with sterile saline. The rabbits were injected (OD received 30 ng or 100 ng of LPS in a 50 µl volume, OS received vehicle) into the mid-vitreous with a BD 300 µL insulin syringe (31G x 5/16 inch needle) inserted through the dorsotemporal quadrant of the eye.

Cell counts and protein

Aqueous humor cell counts were determined manually. Aqueous humor sample was suspended in an equal volume of Turks stain solution, and cell counting performed on a hemocytometer using a light microscope. Protein concentration of the aqueous humor was determined in duplicate using a Pierce® BCA Protein Assay Kit.

MSD Angiogenesis Panel

A commercially available Angiogenisis Panel from Meso Scale Discovery (MSD) was used to test aqueous humor samples of NZW rabbits following intravitreal injections of *E. coli* LPS. The proteins tested using the Angiogenisis Panel is shown in Table 1. A Sector Imager 6000 instrument was used to read plates.

Results

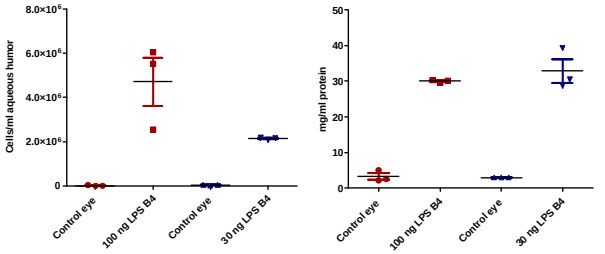
Table 1: Proteins Tested in MSD Angiogenesis Panel

- VEGF
- VEGF-C
- VEGF-D
- VEGFR1
- Placental Growth Factor (PlGF)
- Basic FGF
- Tyrosine kinase-2 (TIE-2)

Optical Exam Findings

- The effect of LPS IVT administration into the OD eye was most pronounced in the conjunctiva (congestion, swelling, and discharge observed in OD eye)
- Examination of the lens, vitreous, and fundus indicated minor findings in the OD eye. No notable findings were observed in the OS eye.
- No abnormalities were noted in cornea, corneal vascularization (i.e., pannus), or during fluorescein staining (i.e., corneal abrasion) for either the OD or OS eye.

Figure 1: Effect of LPS on aqueous humor cell count and protein



Note: A very similar pattern for cell infiltration and increased protein in aqueous humor samples is observed using *E. coli* B5

Figure 2: Kinetics of LPS induced cell infiltration and increased protein levels in aqueous humor

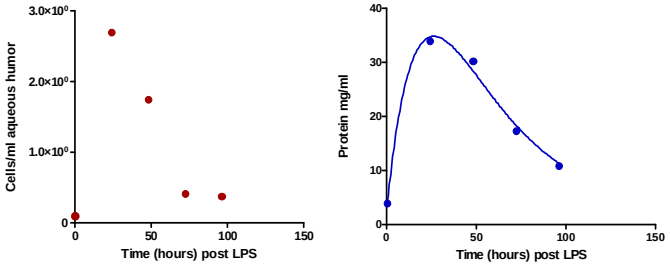


Figure 3: VEGF, VEGF-C, and VEGF-D standard curves

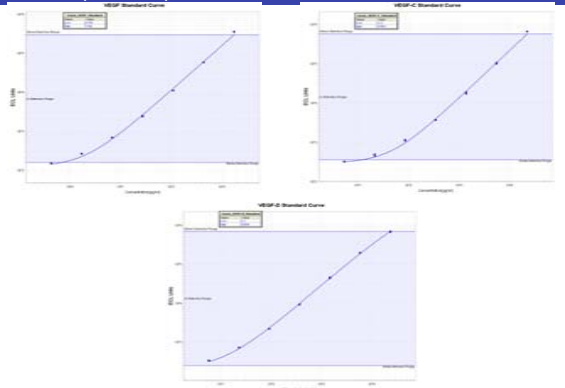
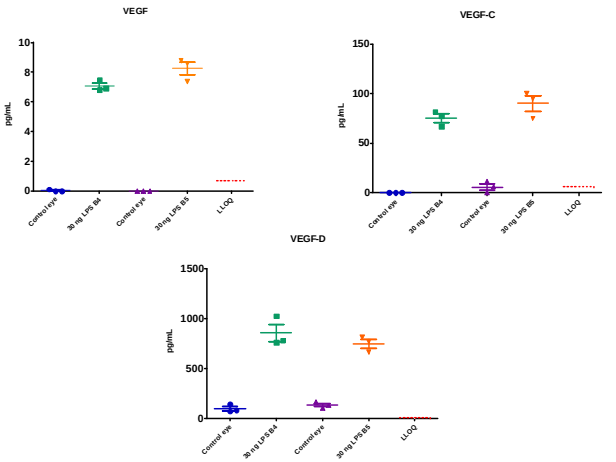


Figure 4: Effect of LPS on VEGF, VEGF-C, and VEGF-D



Conclusions

1. ***E. coli* LPS induces a uveitis in NZW rabbits that results in a large infiltration of leukocytes and significantly increased levels of protein in aqueous humor.**
2. **The induction of uveitis using E. coli LPS (B4 or B5) shows no significant differences between the 30 ng and the 100 ng dosing groups regarding cell infiltration and increased protein levels in aqueous humor. Testing at lower concentrations of LPS may be useful in developing a more sensitive uveitis model for therapeutic**
3. **Maximum cell infiltration and protein increases occur at 24 hours post IVT with treatment.**
4. **Using a commercially available human Angiogenesis Panel from MSD a significant increase in the levels of VEGF, VEGF-C, and VEGF-D are observed in aqueous humor samples from rabbits 24 hours post-intravitreal injection with LPS.**

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