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**Program#/Poster#:** 5270

**Abstract Title:** TLR9 But Not TLR4 Or TLR3 Activation Causes Formation Of Multinucleated Giant Cells And Keratic Precipitates In The Mouse Cornea

**Presentation Start/End Time:** Wednesday, May 09, 2012, 4:00 PM - 4:15 PM

**Session Number:** 472

**Session Title:** Hot Topics in Infection and Immunity

**Location:** Palm A

**Reviewing Code:** 155 corneal immunology and infections - IM

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**Abstract Body:**

**Purpose:** Activation of toll-like receptors (TLRs) on resident corneal macrophages is critical during the early stage of the host inflammatory response to bacterial and viral pathogens. Less is known about the distribution, phenotype and activation status of macrophages in the corneal stroma and endothelium in the later stages of corneal inflammation. We compared the extended macrophage responses in the cornea following exposure to TLR3, TLR4 and TLR9 ligands.

**Methods:** The central corneal epithelium of C57BL/6, Cx3cr1+/gfp, TLR9-/- and CD11c-eYFP mice was debrided and 20ug of TLR3 ligand (Poly I:C), TLR4 ligand (E.coli LPS) or TLR9 ligand (CpG-ODN), sterile saline or control ODN was topically applied. Mice were sacrificed one and four weeks later and corneal whole mounts were immunostained with anti-leukocyte antibodies CD45, CD68, Iba-1 and MHC class II. Macrophages in the corneal stroma and endothelium were analysed by epifluorescence and confocal microscopy.

**Results:** LPS and Poly I:C-induced inflammation led to an increase in stromal macrophages in the central cornea at one week (180 cells/mm² in controls compared to 329 cells/mm² in LPS and 378 cells/mm² in Poly I:C treated corneas). By four weeks, the number of macrophages returned to baseline. The proportion of macrophages expressing MHC class II at one
week increased from 35% in controls to 65% in Poly (I:C) and 48% in LPS-treated corneas. Unlike Poly I:C and LPS-treatment, CpG-ODN led to the accumulation of CD68+Iba-1+MHC class II-CD11c- macrophages on the corneal endothelium. These macrophages formed a syncytium, reflecting clinically observed keratic precipitates. In the stroma of CpG-ODN, but not Poly I:C or LPS-treated corneas, giant multinucleated MHC class II negative macrophages were observed at one and four weeks post-treatment. No giant macrophages or keratic precipitates were observed in corneas from CpG-ODN-treated TLR9-/- mice or in control ODN or saline treated corneas.

**Conclusions:** TLR9 activation by CpG-ODN during corneal inflammation leads to the formation of multinucleated giant stromal macrophages and keratic precipitates on the endothelium. These data suggest that recognition of microbial DNA by TLR9 within host cells may explain chronic macrophage activation following corneal infections.

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