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TLR Ligand-Induced Keratitis Is Partially Reconstituted in TLR–/− Chimeric Mice by Donor TLR+ Bone Marrow-Derived Cells in the Corneal Stroma

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Abstract

**Purpose:** Toll-like receptors (TLRs) are transmembrane proteins that recognise microbial products such as lipopolysaccharide (LPS), a component of the outer wall of gram negative bacteria, and lipoproteins from gram positive bacteria. Upon recognition of LPS (through TLR4) and Pam3cys (through TLR2) TLRs signal to initiate the production of pro-inflammatory cytokines that are essential in the induction of both the innate and adaptive immune responses. Whilst previous studies have shown TLR activation in the mouse cornea during LPS and Pam3cys-induced keratitis, it is still unclear which specific corneal cells (epithelium, stromal, bone-marrow [BM]-derived) express and signal through TLRs. In the present study we sought to determine if the resident BM-derived cells in the mouse cornea are capable of responding to TLR2 and TLR4 ligands.

**Methods:** TLR2–/− and TLR4–/− recipient mice were sub-lethally irradiated reconstituted with BM extracted from donor WT transgenic eGFP mice (TLR+/+). As negative controls, eGFP TLR2–/− and eGFP TLR4–/− mice were also used as donors. At 2-4 weeks post reconstitution, the central 1mm of corneal epithelium was removed using a corneal rust ring remover (Algerbrush) and either 10µg of Pam3cys or 20µg of LPS was applied topically to the area of debridement. Mice were sacrificed 24h later and corneal inflammation was assessed using *in vivo* confocal microscopy, *in vivo* epifluorescence microscopy and immunohistochemistry to measure stromal haze, eGFP+ cell infiltrate and neutrophils (NIMP-R14) respectively.

**Results:** 2 weeks post BM reconstitution 30% of BM-derived stromal cells had been replaced by donor eGFP+ cells. By 4 weeks this had increased to 40% and was 75% by 8 weeks. Upon challenge with TLR
ligands there was a significant increased neutrophil recruitment to the corneal stroma and development of corneal haze in TLR−/− mice reconstituted with C57Bl/6 BM cells compared with TLR−/− mice reconstituted with TLR+/+ BM cells. However, reconstitution of LPS and Pam3cys-mediated corneal inflammatory response in these mice was less than for C57Bl/6 mice reconstituted with C57Bl/6 BM cells.

**Conclusion:** Results of this study demonstrated that the presence of TLR4+ and TLR2+ BM-derived cells in TLR4−/− and TLR2−/− mouse corneas is sufficient to initiate a response to LPS and Pam3cys-induced keratitis. However, as the response of TLR−/− mice reconstituted with C56Bl/6 BM was lower than that for wild-type mice, there may also be a contribution of non-hematopoietic corneal keratocytes and epithelial cells.

**Keywords:** cornea: basic science • inflammation • keratitis

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