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Abstract

**Purpose:** The cornea contains extensive populations of dendritic cells (DCs) in both the epithelium and the stroma, as well as heterogeneous populations of stromal macrophages; however, little is known about the function of these cells in the naive and inflamed cornea. The chemokine receptor Cx3cr1 governs the homeostatic recruitment of DCs to the mouse corneal epithelium, but plays no role in the homing of macrophages to the stroma. We used Cx3cr1-deficient mice, which lack MHC Class II DCs in the corneal epithelium, to investigate the role for intraepithelial DCs during the innate inflammatory response to the TLR4 ligand LPS.

**Methods:** Wild-type (WT), Cx3cr1 heterozygous (which have one functional copy of the Cx3cr1 gene) and Cx3cr1 homozygous (Cx3cr1-deficient) mice aged 6-12 weeks on both a BALB/c and C57Bl/6 background were used. The central corneal epithelium was partially debrided and 20µg of Ultrapure LPS (TLR4-specific) or sterile saline was applied to the cornea. 24 or 72 hours later eyes were either enucleated and frozen or fixed in 4% paraformaldehyde. For quantitation of infiltrating neutrophils and macrophages, frozen sections were cut (6µm) and immunostained with NIMP (neutrophils) or F4/80 (macrophages). To quantify epithelial DCs in the healthy and inflamed corneal epithelium, corneal wholemounts were immunostained with M5/114 antibody and MHC Class II+ cells were counted by epifluorescence microscopy.

**Results:** The density of epithelial DCs was unchanged in both WT and Cx3cr1-deficient mice at both 24 and 72 hours when compared to the density of DCs in the resting corneal epithelium. Compared with wild type mice, Cx3cr1-deficient mice displayed significantly fewer neutrophils in the cornea 24 hours following LPS-keratitis (BALB/c, p=0.006, C57Bl/6; p=0.001). Cx3cr1 heterozygous mice, which have one functional copy of Cx3cr1, also displayed significantly fewer infiltrating neutrophils when compared to WT mice (BALB/c; p<0.01, C57Bl/6; p=0.01), however this inhibited response was not as great as Cx3cr1-deficient mice. In all strains of mice examined, there was no significant difference in macrophage recruitment following LPS-induced keratitis depending on Cx3cr1 expression.

**Conclusion:** The absence of epithelial DCs in the mouse cornea may not affect LPS-responsiveness, however a functional deficiency of one or both alleles of Cx3cr1 may impair TLR4 signaling by Cx3cr1 positive cells in the mouse cornea.