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## CX<sub>3</sub>CR1-Dependent Homing of MHC Class II Positive Cells to the Normal Mouse Corneal Epithelium

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### Abstract

**Purpose:** The cornea was traditionally thought to be devoid of resident immune cells. Recent investigations however have confirmed that there are in fact populations of macrophages and dendritic cells (DCs) in the stroma and epithelium of the cornea, although the precise phenotype and distribution are still controversial. CX<sub>3</sub>CR1, the sole receptor for the chemokine fractalkine, is expressed by these monocyte-derived cells.

We have utilized transgenic CX<sub>3</sub>CR1<sup>GFP</sup> mice, in which either one (heterozygous) or both (homozygous) copies of the CX<sub>3</sub>CR1 gene were replaced by green fluorescent protein (eGFP), to characterise monocyte-derived cells in the mouse cornea.

**Methods:** Wholemount corneas from naïve 6-12 week old mice were immunostained with leukocyte antibodies MHC class II, CD169, CD11b and CD68 and analysed using epifluorescence and confocal microscopy. MHC class II<sup>+</sup> cells were counted in the corneal epithelium of wild-type, CX<sub>3</sub>CR1<sup>+/GFP</sup> heterozygous and CX<sub>3</sub>CR1<sup>GFP/GFP</sup> homozygous mice.

**Results:** We found a significant reduction in the number of MHC class II<sup>+</sup> cells (putative DCs) in the corneal epithelium of CX<sub>3</sub>CR1-deficient mice (p<0.009) compared to wild-type mice. Throughout the thickness of the corneal stroma, immunostaining revealed GFP<sup>+</sup> cells that were double positive for either CD169, CD68, CD11b or MHC class II indicating a macrophage phenotype, in both heterozygous and

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homozygous (CX<sub>3</sub>CR1-deficient) mice. These were also distributed both centrally and peripherally.

**Conclusions:** This study clearly demonstrates a critical role for fractalkine and its receptor, CX<sub>3</sub>CR1, in the normal homing of MHC class II<sup>+</sup> cells to the corneal epithelium.

**Keywords:** cornea: epithelium • keratitis • inflammation



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