The role of intraepithelial CX3CR1^{hi} macrophages in the immune regulation of the murine epididymis

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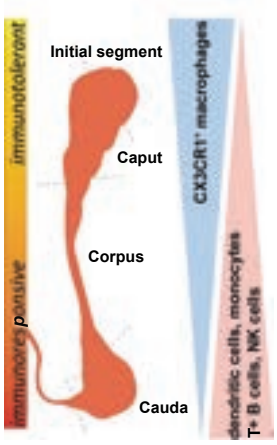
Background

The epididymis faces contrasting immunological challenges, i.e. tolerance towards maturing spermatozoa vs. immune reactivity against pathogens. Accordingly, the opposing ends of the epididymal duct exhibit different immune responses upon bacterial infection. We have previously shown that resident immune cells are strategically positioned along the epididymal duct to shape distinct immunological environments. CX3CR1^{+} macrophages constitute the major resident immune cells population and show region-specific specializations. Based on their canonical function (tissue homeostasis and regulation of immune responses), we hypothesize that CX3CR1^{+} macrophages play a crucial role in epididymal immune regulation.

This study aims at depleting tissue-resident CX3CR1^{+} macrophage using *Cx3cr1*^{CreERT2}*Rosa26*^{iDTR} mice to analyze the phenotypical consequences under physiological and pathological conditions.



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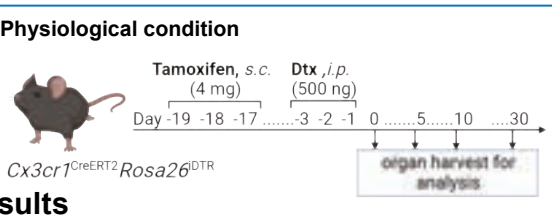
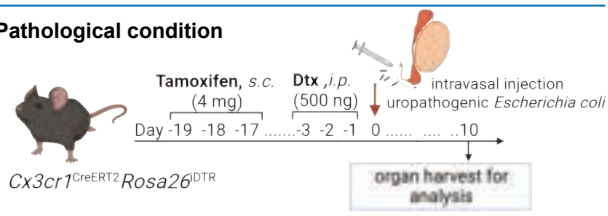
Methods





Results

Targeted depletion of intraepithelial CX3CR1^{+} macrophages results in focal epithelial damage under physiological conditions.

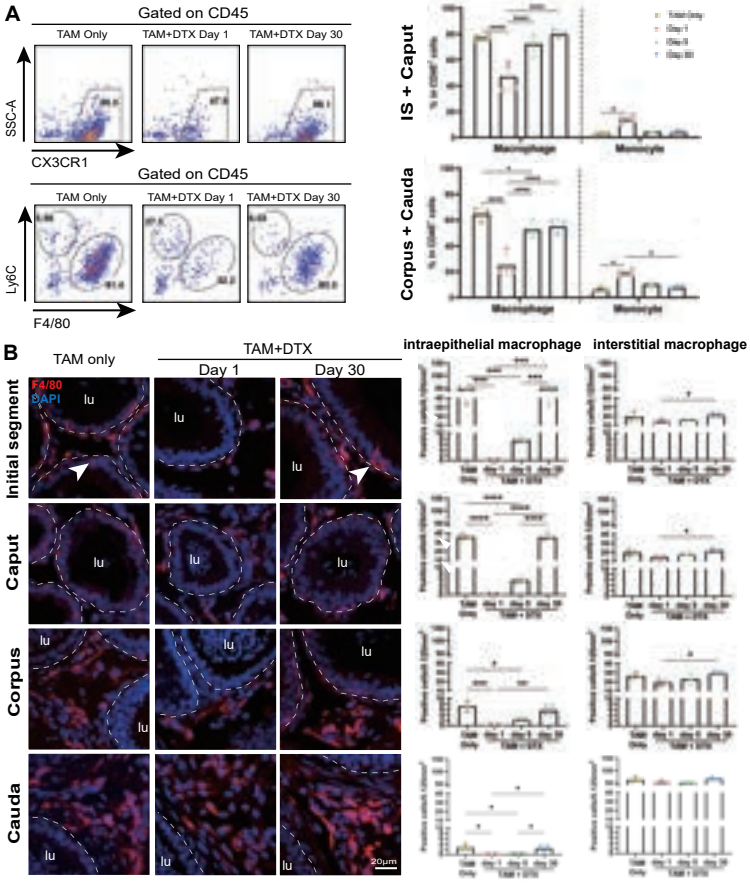


Figure 1: **Specific depletion of intraepithelial CX3CR1^{hi} macrophages along the epididymis in *Cx3cr1*^{CreERT2}*Rosa26*^{iDTR} mice**. (A) Flow cytometry analysis of macrophages (F4/80^{+}CD11b^{+}Ly6C^{-}) and monocytes (F4/80^{-}CD11b^{+}Ly6C^{+}) in proximal and distal epididymal regions. (n=3-4, mean ± SD). (B) Immunofluorescence staining of F4/80^{+} cells (red) in epididymal regions with semi-quantitative assessment by counting F4/80^{+} macrophage in the epithelial and interstitial compartment (n=3-5, mean ± SD).

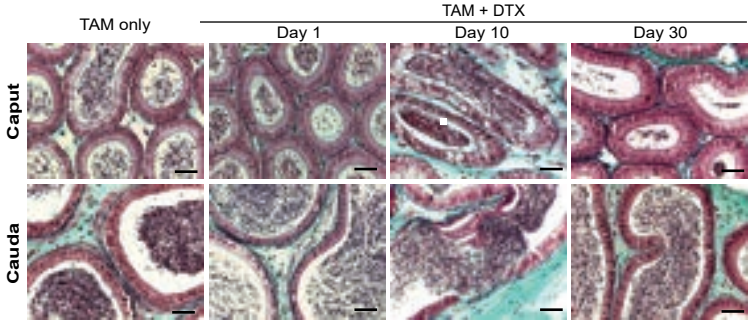


Figure 2: Histological alterations in *Cx3cr1*^{CreERT2}*Rosa26*^{iDTR} mice. Representative images show focal epithelial damage in caput and the extravasation of spermatozoa in cauda of macrophage-depleted mice at day 10, that recovered after 30 days. Scale bar 50 µm. (Masson-Goldner-Trichrome staining)

Mice lacking intraepithelial CX3CR1^{hi} macrophages develop more severe immune reaction within the proximal regions upon bacterial infection.

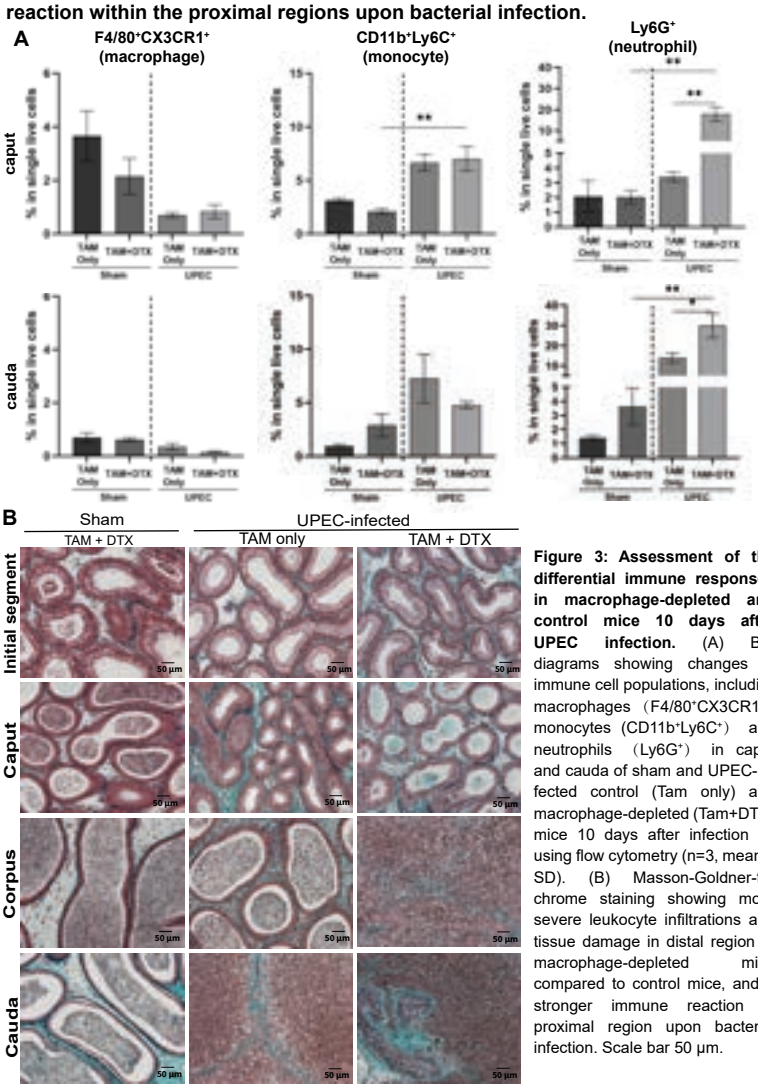


Figure 3: Assessment of the differential immune responses in macrophage-depleted and control mice 10 days after UPEC infection. (A) Bar diagrams showing changes in immune cell populations, including macrophages (F4/80^{+}CX3CR1^{+}), monocytes (CD11b^{+}Ly6C^{+}) and neutrophils (Ly6G^{+}) in caput and cauda of sham and UPEC-infected control (Tam only) and macrophage-depleted (Tam+DTX) mice 10 days after infection by using flow cytometry (n=3, mean ± SD). (B) Masson-Goldner-trichrome staining showing more severe leukocyte infiltrations and tissue damage in distal region of macrophage-depleted mice compared to control mice, and a stronger immune reaction in proximal region upon bacterial infection. Scale bar 50 µm.

Conclusion and Outlook

We successfully established a mouse model for targeted depletion of intraepithelial CX3CR1^{+} macrophages within the epididymis allowing a comprehensive assessment of their function in immune homeostasis and defense within future studies. So far, our data suggest a pivotal role of CX3CR1^{+} macrophages in maintaining epithelial integrity required for propel sperm maturation and in controlling the magnitude of immune response.

We will assess in future approaches the impact of macrophage depletion on sperm maturation as well as on disease progression.

Financial support was received from the von-Behring-Roentgen-Foundation (Marburg, Germany) and German Society of Andrology (DGA).

Pleuger C, Ai D, Hoppe M L, et al. The regional distribution of resident immune cells shapes distinct immunological environments along the murine epididymis. Elife, 2022,11.