

Investigating the role amphiregulin in kidney defence and repair

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Background: Urinary tract infections (UTI) are common and cause significant morbidity¹. Most infections are caused by uropathogenic Escherichia coli (UPEC) and recurrent pyelonephritis can lead to scarring and chronic kidney disease¹.

Tissue resident immune cells, including mononuclear phagocytes (MNs) play an important role in defence against infections by internalising invading bacteria and recruiting neutrophils^{2 3}. In addition, they may promote epithelial integrity and repair, for example by secreting the epidermal growth factor receptor ligand Amphiregulin (AREG)⁴. AREG has been shown in a number of models to be critical for both inflammation and repair however little is known of its role in the kidney⁵.

Aims: To determine AREG expression in human and mouse kidney, including the anatomical distribution and cellular source. To ascertain whether AREG plays a role in defence against UTI and in susceptibility to kidney fibrosis.

Methods: Human kidney samples were processed for bulk⁸ and single cell RNA sequencing^{9 10}. In vitro studies were performed using an immortalised human proximal tubule cell line (HK-2) and monocyte-derived macrophages (MDM). An in vivo model of acute and chronic UTI was performed in C57BL/6 (WT) or Areg^{-/-} mice.

Results: In human kidneys we observed higher AREG expression in the medulla compared to the cortex. Single cell RNA sequencing demonstrated that MNs in both human and murine kidneys expressed AREG and this was confirmed by flow cytometric analysis. In vitro MDMs secreted AREG following exposure to UPEC. Recombinant AREG in vitro promoted both HK2 survival and repair in the presence of UPEC. In vivo, Areg^{-/-} mice had increased susceptibility to pyelonephritis with reduced numbers of polymorphonuclear neutrophils, newly recruited Ly6C^{hi} / MHCII^{lo} monocytes and transitioning Ly6C^{int} / MHCII^{int} macrophages compared to controls. RT-qPCR of infected kidneys revealed reduced Ccl2 transcripts in Areg^{-/-} mice.

In recurrent / chronic UTI, WT mice demonstrated higher transcripts of Areg and fibrosis-associated genes Col1a1 and Fn1 compared to controls; these were also highest in the medulla/pelvis region. Areg^{-/-} mice showed less fibrosis (p<0.001) compared to controls by Sirius Red staining and RTqPCR following chronic UTI. Areg^{-/-}→WT bone marrow chimeras also demonstrated less fibrosis compared to WT^{-/-}→WT.

Conclusion: AREG expression is highest in the renal medulla and MNs are a major source of AREG. Our data show that AREG contributes to defence against pyelonephritis in vivo, and this may be in part by promoting circulating monocytes recruitment to the kidney by enhancing Ccl2-production by epithelial cells. AREG production as well as fibrosis related genes are increased in the context of UTI-induced kidney fibrosis and these findings are abrogated in Areg^{-/-} animals. While experiments are still ongoing AREG may potentially offer a future therapeutic target in CKD.