**Spatial RNA sequencing and mass cytometry identify estrogen-dependent control of neutrophils activation as a protective mechanism in renal ischemia-reperfusion injury**

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**Background.** Previous experimental and clinical studies have highlighted sex-specific susceptibility to ischemia-reperfusion injury (IRI) in multiple organs, with evidence underlying the potential protective effect of female sexual hormones. Nevertheless, their precise effect in the regulation of immune responses and tissue inflammation following IRI still needs to be investigated.

**Methods.** Renal IRI was modelled in mice using unilateral nephrectomy, followed by 23min ischemia and reperfusion. Renal function was evaluated by transcutaneous assessment of FITC-Sinistrin clearance. Visium spatial mRNA sequencing was carried out on paraffin-embedded tissue. Mass and multiparameter flow cytometry was applied on peripheral blood mononuclear cells.

**Results.** Following renal IRI, females had a significantly better glomerular filtration rate and reduced tubulointerstitial lesions compared to age-matched males. Besides the C57BL/6-based initial experimental model, these results were reproducible in various genetically distinct mouse strains. This protection was alleviated after ovariectomy. Spatial mRNA sequencing revealed a differential transcriptomic profile in male and female proximal tubular cells with an upregulation of genes associated with failed damage repair in males. Mass cytometry identified neutrophils as the primarily recruited immune cells in males peripheral blood and in injured renal tissue. *In vivo* depletion of neutrophils with a specific anti-Ly6G monoclonal antibody reduced IRI in males.

**Conclusions.** Our data showed that female mice were protected from renal IRI independently of genetic background and in an estrogen-dependent manner. Spatial transcriptomics and mass cytometry allowed us to broadly characterize sex-specific tissular gene expression and systemic immune responses following renal IRI. Males proximal tubular cells were shown to have impaired damage repair associated with increased neutrophils recruitment. Sex-specific depletion of neutrophils reduced renal IRI. Overall, our data suggest that neutrophils are necessary in the pathophysiology of IRI and could be targeted, *per se* or via their effector function, to reduce early damage and ameliorate organ preservation for solid organ transplantation.