Soluble urokinase plasminogen activator receptor (suPAR) inflames kidneys with T cells and aggravates sepsis-induced acute kidney injury

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Introduction

The soluble urokinase plasminogen activator receptor (suPAR) is an immune-derived glycoprotein implicated in the pathogenesis of acute kidney injury (AKI). Sepsis is a strong inducer of plasma suPAR levels and a known contributor to the development of AKI. We hypothesized that suPAR is directly involved in the pathophysiology of sepsis-induced AKI.

Methods and Materials

We used a polymicrobial model of sepsis in wild-type (WT), uPAR knockout (KO, suPAR deficient), and transgenic suPAR-overexpressing (OE) mice. We compared measures of kidney function, tissue damage, and tissue inflammation in septic and untreated mice. Kidney tissue inflammation was quantified by kidney flow cytometry, immunohistostaining, and kidney luminex assay.

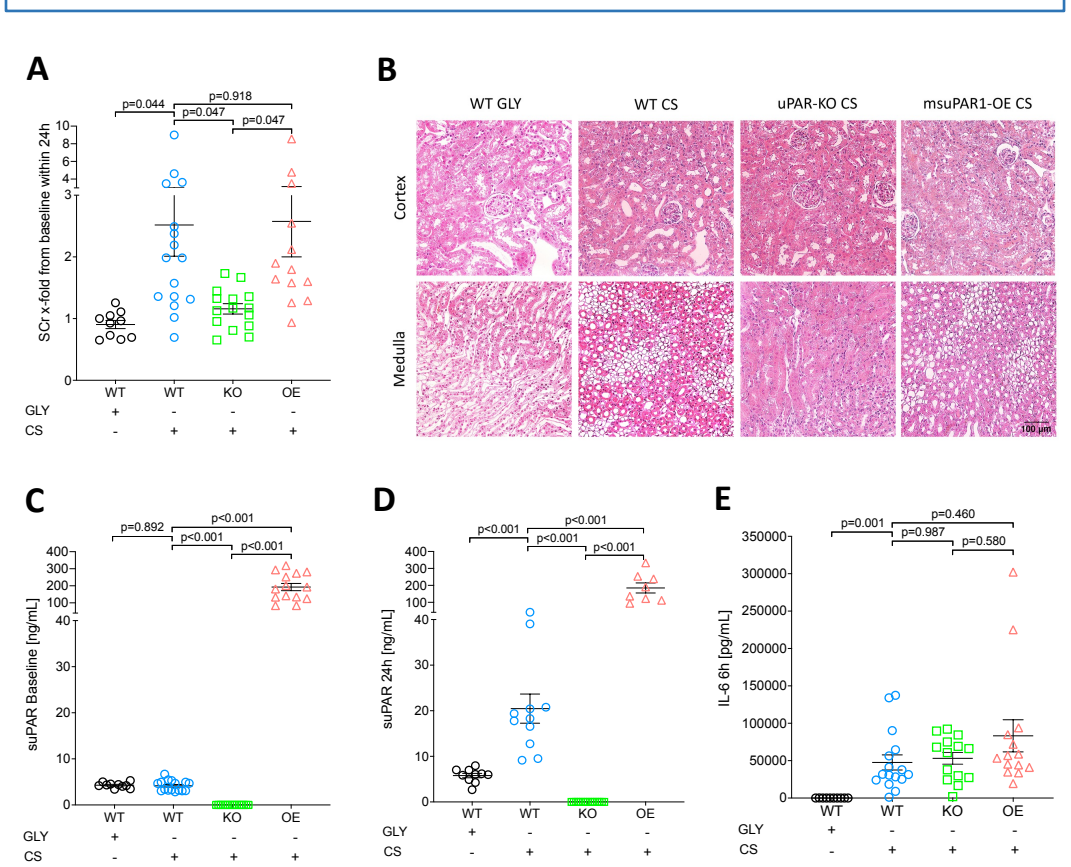

Figure 1. Elevated blood levels of suPAR are associated with enhanced kidney tissue damage and kidney function impairment. Sepsis was induced via i.p. injection of 250 µL cecal slurry (CS) in C57BL/6 wild-type (WT, n=16), urokinase plasminogen activator receptor knockout (KO, n=15) and transgenic C57BL/6 with overexpression of suPAR (OE, n=14). 15% glycerol (GLY) served as control in WT (vehicle control, n=10). **(A)** Maximum serum creatinine (SCr) changes from baseline within 24h. **(B)** HE staining of kidneys from different mouse strains 24h after sepsis induction (40x). **(C)** suPAR levels at baseline and **(D)** 24h after sepsis induction, and (E) interleukin-6 (IL-6) levels 6h after sepsis induction. Data are reported as mean (standard error of the mean).

Results

Kidneys from untreated OE mice expressed high levels of interleukin-16 (IL-16) and C-C motif chemokine ligand 3 (CCL3); both involved in cell-mediated kidney injury and potent chemo-attractants for T and NK cells. Consistent with this expression pattern, we found significantly increased numbers of kidney T and NK cells in untreated OE mice, equaling numbers observed in septic WT mice. Further, high plasma suPAR aggravated sepsis-induced ultrastructural kidney damage, kidney tissue inflammation and kidney function impairment after 24h of sepsis. In contrast, KO mice showed a strong protective effect against AKI. Kaplan-Meier analysis revealed a survival benefit of KO over OE mice (87% vs. 50%, p=0.033). The composition of kidney immune cells in sepsis was strongly influenced by varying suPAR plasma levels. In contrast to kidney neutrophils, numbers of kidney T cells were strongly linked to the extent of systemic suPAR elevation and kidney function impairment, with significant higher numbers in septic OE mice compared to septic WT and KO mice.

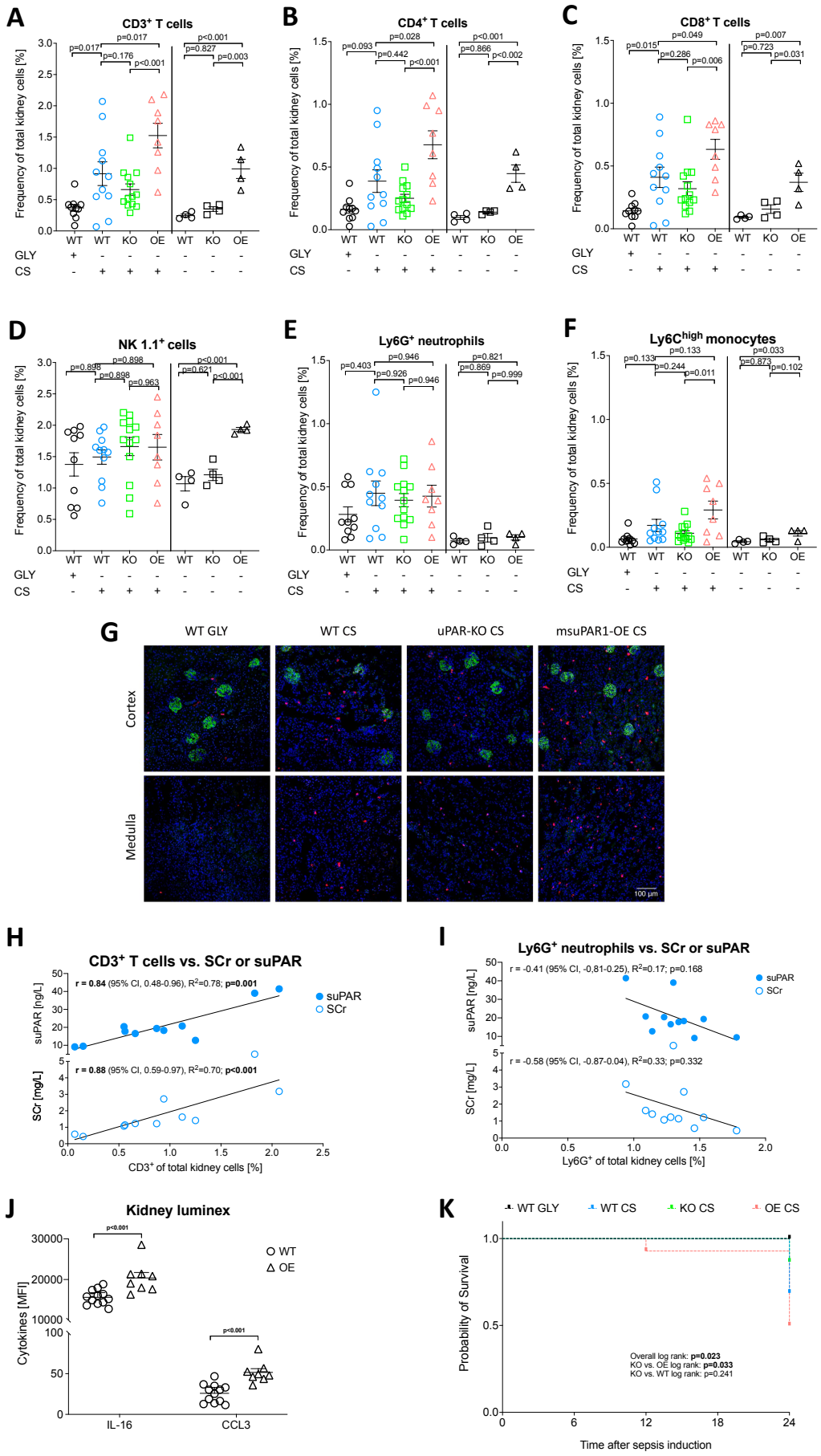

Figure 2. Characterization of kidney leukocyte subsets in C57BL/6 wild-type (WT), urokinase plasminogen activator receptor knockout (KO) and transgenic C57BL/6 with overexpression of suPAR (OE) reveals a link of increased blood suPAR levels with kidney T & NK cell accumulation, kidney function impairment, local upregulation of inflammatory cytokines and poor outcome in murine sepsis. **(A-F)** Strain-dependent characterization of leukocyte subsets by kidney flow cytometry after 24h of sepsis induction (left part) via i.p. injection of 250µL cecal slurry (CS) and untreated mice (right part). Injection of 15% glycerol (GLY) served as control (vehicle solution). **(G)** Exemplary double immunofluorescence staining (40x) for podocin (green) and CD8+ T cells (red) of kidney tissue from different mouse strains after 24h of sepsis. **(H+I)** Correlation analysis of kidney T cells and neutrophils with corresponding blood serum creatinine (SCr) and suPAR levels in WT sepsis. **(J)** Kidney Luminex analysis of homogenized kidney tissue of untreated WT and OE mice. **(K)** Survival analysis of different mouse strains. Survival: WT GLY 10/10 (100%), WT CS 11/16 (69%), KO CS 13/15 (87%), OE CS 7/14 (50%). Data are reported as mean (standard error of the mean).

Conclusions

SuPAR inflames the kidney with T cells potentially via local upregulation of IL-16 and CCL3. "SuPAR inflamed" kidneys react with increased kidney injury in sepsis which can potentially be improved by targeting the immune system or deleting suPAR. These findings hold great potential for new therapeutic strategies.

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