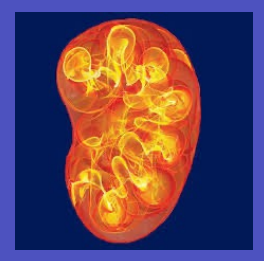
A macrophage-endothelial immunoregulatory axis ameliorates septic acute kidney injury

Jamie R. Privratsky[1,2], Shintaro Ide[3], Yanting Chen[1], Hiroki Kitai[3], Jiafa Ren[3], Helene Fradin-Kirshner[4], Xiaohan Lu[3], Tomokazu Souma[3], Steven D. Crowley[3,5]

[1]Center for Perioperative Organ Protection, [2]Division of Critical Care Medicine, Department of Anesthesiology, Duke University Medical Center, Durham, NC
[3]Division of Nephrology, Department of Medicine, Duke University Medical Center, Durham, NC
[4]Duke Center for Genomic and Computational Biology, Duke University Medical Center, Durham, NC
[5]Durham VA Medical Center, Durham, NC

Duke Anesthesiology
Duke University School of Medicine



Abstract

Purpose: The most common cause of acute kidney injury (AKI) in critically ill patients is sepsis. Kidney macrophages consist of both F4/80^{hi} and CD11b^{hi} cells. The role of macrophage subpopulations in septic acute kidney injury pathogenesis remains unclear. As F4/80^{hi} macrophages are reported to contribute to immunomodulation following injury, we hypothesized that F4/80^{hi} macrophages can limit septic acute kidney injury.

Methods: F4/80hi macrophages were depleted via diphtheria toxin injection in CD11cCre(+)/CX3CR1dtr/wt (F4/80 MKO mice) compared to CD11cCre(-)/CX3CR1dtr/wt (F4/80 MWT) mice. F4/80 MWT and F4/80 MKO mice were subjected to sham or cecal ligation and puncture (CLP) sepsis. Kidney injury was assessed by serum creatinine and blood urea nitrogen, histologic injury scoring, and KIM-1 staining by immunofluorescence microscopy. Cytokine mRNA and protein levels were measured by RT-PCR and ELISA. Fluorescent cell-sorting and single cell RNA sequencing with Nichenet analysis were used to profile gene expression and identify cell-specific ligand-receptor interactions following CLP in intra-renal cell lineages.

Results: Compared to F4/80 MWT mice, F4/80 MKO mice displayed worsened septic acute kidney injury at 24 hours as measured by serum creatinine (mean ± SD: 0.17 ±0.08 vs 0.41 ± 0.17 mg/dl, p<0.001), histologic injury scoring, and KIM-1 staining. F4/80 MKO kidneys elaborated higher interleukin-6 levels, specifically from kidney endothelial cells. Mechanistically, single cell RNA sequencing and fluorescent cell sorting identified a macrophage-endothelial cell immunoregulatory axis that underlies interleukin-6 expression: F4/80^{hi} macrophages expressed interleukin-1 receptor antagonist that limited interleukin-6 expression in endothelial cells. In turn, both recombinant human interleukin 1 receptor antagonist and anti-interleukin-6 therapy ameliorated septic acute kidney injury in F4/80 MKO mice.

Conclusions: F4/80^{hi} macrophages express interleukin-1 receptor antagonist and constrain interleukin-6 generation from endothelial cells to limit septic AKI, representing a targetable cellular crosstalk in septic AKI.

Introduction

- Kidney macrophages
  - Consist of both F4/80^{hi} and CD11b^{hi} subtypes
  - Can have divergent effects depending on the type of AKI *(Lever JM, et al. JCI Insight, 2019; Salei N, et al. J Am Soc Nephrol, 2020; Zimmerman KA, et al. J Am Soc Nephrol, 2019; Salei N, et al. PNAS, 2021)*
- Septic acute kidney injury (AKI)
  - Sepsis is the most common cause of AKI *(Zarbock, et al. Curr Opin Crit Care, 2014)*

What roles do macrophages populations play during development of septic AKI?

Hypothesis: Kidney macrophages subpopulations will have divergent effects during septic AKI

Results

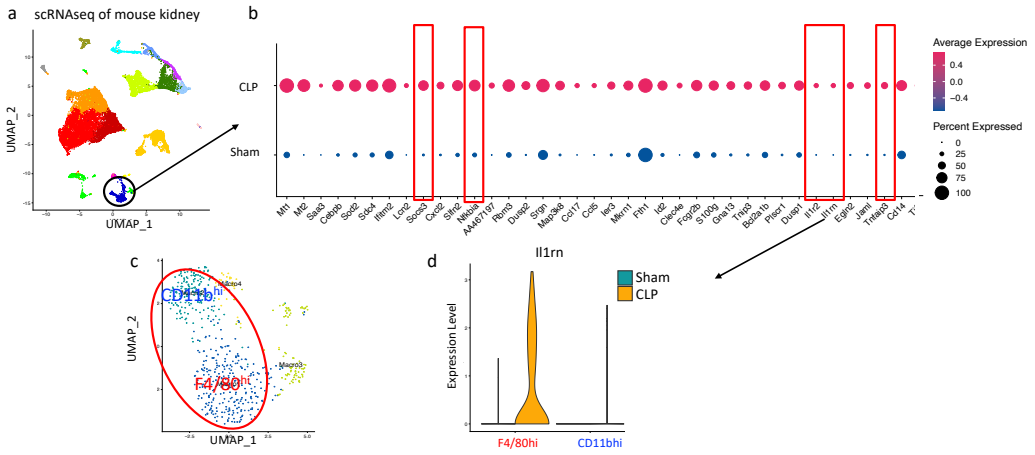


Figure 1. ***Kidney macrophages upregulate anti-inflammatory genes following sepsis including interleukin 1 receptor antagonist (IL1ra/Il1rn).*** Mice were subjected to sham surgery or cecal ligation and puncture (CLP) surgery. At 24 hours after surgery, kidneys were harvested. (a) single cell RNA sequencing analysis was performed with UMAP plot showing cell clusters in kidney (circle denotes macrophage cluster). (b) Top 50 upregulated genes following CLP in macrophage cluster by differential expression analysis. Notice upregulation of anti-inflammatory genes including *Socs3, Tnfaip3*, and Il1-pathway antagonists *Il1rn* and *Il1r2*. (c) (a) UMAP plot demonstrating 4 separate cell clusters within macrophage population after subclustering. (d) Violin plots displaying expression of *Il1rn* in F4/80hi and CD11bhi based on experimental group.

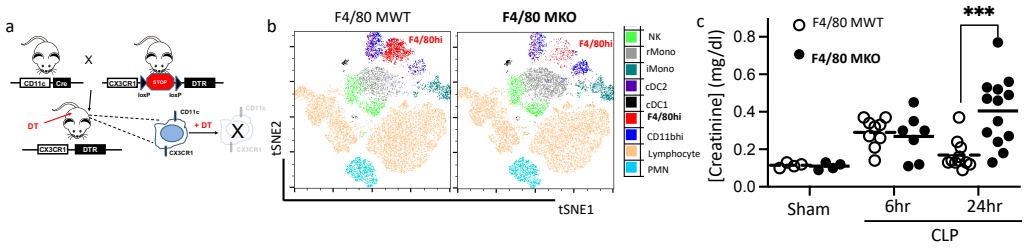


Figure 2. Selective depletion of F4/80hi macrophages in F4/80hi macrophage knockout (F4/80 MKO) mice worsens septic AKI. (a) Genetic strategy to deplete F4/80hi macrophages. (b) Mice were administered diphtheria toxin for three successive days and then flow cytometric analysis was performed one day later on kidney cell digests. tSNE plot of myeloid populations within kidney demonstrating depletion of F4/80hi population in F4/80 MKO mice compared to littermate control F4/80 MWT mice. (c) Serum creatinine was measured at each indicated timepoint following cecal ligation and puncture (CLP) sepsis. Dots in graph show individual samples with line at mean of group. F4/80 MKO mice displayed higher levels of serum creatinine at 24 hours following procedure (n=4 for sham groups, n=7-9 mice for 6hr groups, n=11-13 for 24hr groups, ***p<0.001 as determined by two-way ANOVA).

Results

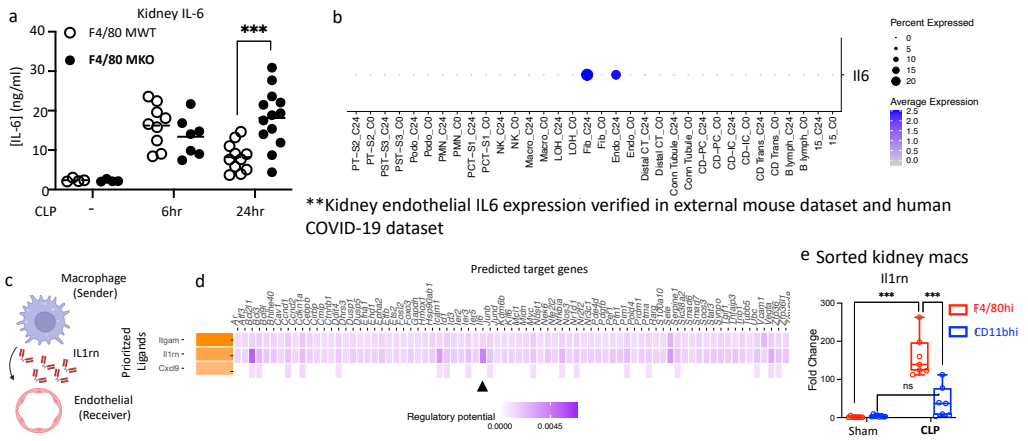


Figure 3. Il6 is produced by endothelial cells after sepsis and induces anti-inflammatory signaling in F4/80hi macrophages. (a) Levels of IL-6 as measured by ELISA in whole kidney tissue in F4/80 MWT and F4/80 MKO mice after sham or CLP. Dots represent individual mice (procedure (n=4 for sham groups, n=7-9 mice for 6hr groups, n=11-13 for 24hr groups). Significance determined by two-way ANOVA (***p<0.001). (b) Wildtype mice were subjected to sham or CLP. At 24 hrs, kidneys were harvested and subjected to scRNAseq analysis. Dot plot showing expression of Il6 in each cell cluster between sham (C0) and CLP (C24) mice. Note the limited expression of Il6 in all cell types except endothelial cells and fibroblasts after CLP. (c) Schematic of Nichenet analysis ligand-receptor interaction of single cell RNA sequencing demonstrating endothelial cells as sender and macrophages as receiver (figure created with BioRender). (d) Nichenet analysis of top 3 prioritized ligands secreted from endothelial cells and downstream target genes in macrophages. Arrowheads indicate anti-inflammatory genes of interest. (e) Kidneys were harvested and fluorescent cell sorting was performed. RT-PCR was performed on sorted cell populations. Boxplots show fold change in mRNA expression of *Il1rn* for each group compared to Sham F4/80hi (Line at median, error bars show minimum and maximum values for each group). Dots represent individual samples for each group (n = 8 for sham, n = 7 for CLP). Statistical analysis performed by two-way ANOVA (ns-not significant, **p<0.01, ***p<0.001).

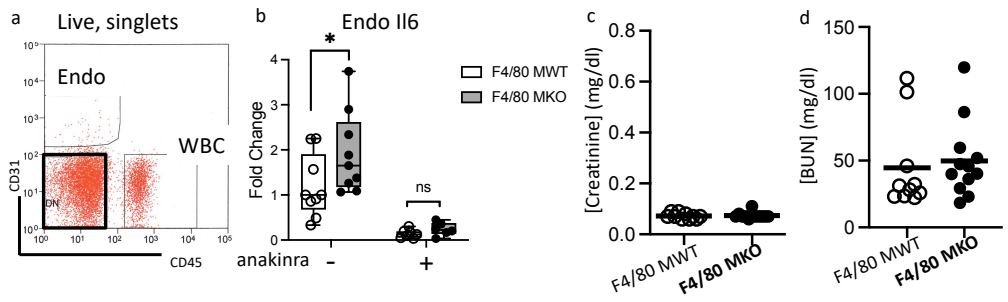


Figure 4. F4/80hi macrophages limit IL6 generation from endothelial cells and anti-IL6 therapy amelioriates septic AKI. (a,b) RT-PCR was performed on sorted endothelial cells from F4/80 MWT and F4/80 MKO mice after CLP. (a) Fluorescent dot plot demonstrating expression of CD31 and CD45 in Live, singlets. Endo gate denotes endothelial cells. WBC denotes white blood cells (CD45+). (b) RT-PCR for *Il6* mRNA expression was performed on sorted cells from Endo gate in (a). Each dot within boxplot denotes individual sample. Significance determined by Mann-Whitney test (*p<0.05). (c,d) F4/80 MWT and F4/80 MKO mice were subjected to CLP. Immediately after CLP, mice were injected with anti-IL-6 antibody (1.33 mg/kg). At 24 hours, mice were sacrificed. Serum (c) creatinine and (d) blood urea nitrogen (BUN) were measured.

Conclusions

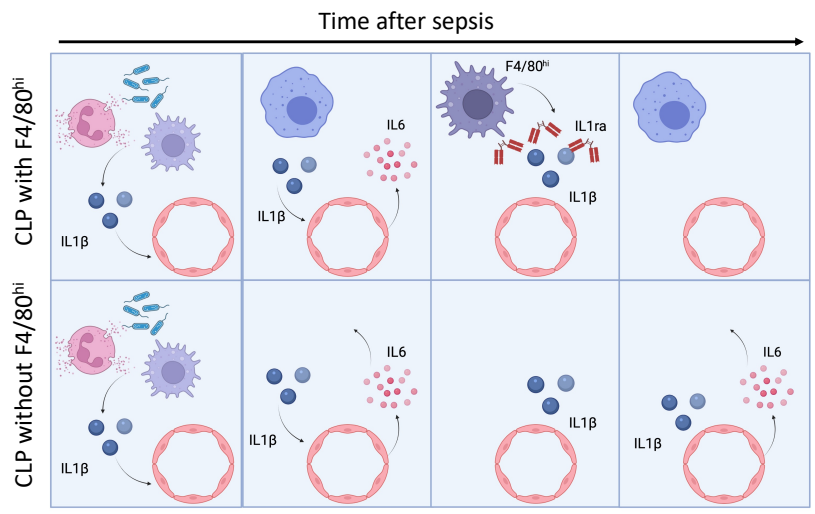


Figure 5. Schematic of working model postulating that F4/80hi macrophages limit IL6 generation from endothelial cells to ameliorate septic AKI (figure created with BioRender).

THE 28TH INTERNATIONAL CONFERENCE ON
ADVANCES IN CRITICAL CARE NEPHROLOGY
AKI & CRRT 2023
MARCH 29 - APRIL 1 SAN DIEGO, CALIFORNIA