Gut microbiota-immune system interactions during acute kidney injury

Sanjeev Noel¹, Fuad Mohammad¹, James White², Kyungho Lee¹, Sepideh Gharaie¹, Hamid Rabb¹

¹Department of Medicine, Johns Hopkins University, Baltimore, MD, United States
²Resphera Biosciences, Baltimore, MD, United States

Corresponding Author

Hamid Rabb, MD
Division of Nephrology, Department of Medicine, Johns Hopkins University, Ross 965
720 Rutland Avenue, Baltimore, Maryland 21205
Phone: 410-955-5268
Email: hrabb1@jhmi.edu
**Gut microbiota-kidney cross talk**

Distant organ cross-talk during acute kidney injury (AKI), first studied mechanistically in the lung (1), is now established to play an important role in disease outcomes. The relationship between intestinal microbiota and AKI has also been explored with exciting findings that are promising for future human therapeutics. In search of ways to reduce kidney T cells by using “cleaner”, germ-free (GF) mice, it was unexpectedly found that GF mice not only retained their kidney immune cells but had a higher number of natural killer T (NKT) cells and reduced interleukin (IL)-4 levels compared to normal wild type (WT) mice. Moreover, GF mice subjected to kidney ischemia reperfusion (IR) injury had increased CD8 T cell trafficking and inflammatory cytokine mediators compared to normal WT mice (2). Induction of ischemic AKI in GF mice led to worse course of AKI in terms of both structure and function. Conventionalizing GF mice with normal mouse stool led to normalizing T cell and NKT populations, and relative protection from AKI compared to GF mice (2). It was subsequently observed that early exposure to gut microbiota during development was associated with long-term changes in NKT cell function in non-renal tissues and NKT cells in GF mice had a less mature phenotype with diminished activation capacity upon antigen encounter (3, 4). To elucidate whether the effects were bidirectional and AKI led to changes in gut microbiome, gut microbiota was evaluated in C57BL/6 WT mice after ischemic or nephrotoxic (cisplatin-CP) injury. DNA was isolated from the fecal pellets (n=4-5 per group), at baseline (D0) and 3 days (D3) post IR. V3-V5 region of 16S rRNA gene was amplified using 357F/926R primer set and sequenced using the Roche/454 platform. IR and CP modified relative abundance of specific bacterial species belonging to phylum Actinobacteria,
Bacteroidetes, Firmicutes, Tenericutes and Verrucomicrobia (Fig 1A). Furthermore, differential abundance testing and negative binomial testing showed distinct alterations in microbial populations at family and genus level respectively (Fig 1B). Major bacterial families affected after CP injections included Lachnospiraceae, Lactobacilaceae, Porphyromondaceae and Ruminococcaceae while Erysipelotrichaceae, Lachnospiraceae, Porphyromondaceae and Ruminococcaceae were changed by IR. At the genus level, Oscillibacter, Lactobacillus, Clostridium and Barnesiella changed after CP induced AKI and Oscillibacter, Eisenbergiella and Barnesiella changed after IR induced AKI. Dimensional analysis by Brey NMDS and alpha diversity analysis using Shannon index revealed that microbiome differentiated over time depending on treatment (Fig 1C&D). Further analysis showed significant increase in the percentage of Erysipelotrichiaceae insertae sedis in post-AKI (D3) samples compared to baseline (D0) samples from IRI (p=0.03) and CP (p=0.007) treated mice. Conversely, the percentage of Lactobacillus decreased significantly (p=0.02) at D3 after CP treatment in comparison to D0 samples (Fig 1E).

Gut microbiota, amino acids, antibiotics and AKI

Further studies have elegantly examined AKI and microbiome interactions. Worse outcome of AKI in GF mice was confirmed in a study that associated AKI-induced gut dysbiosis with reduced D–amino acid oxidase activity and altered D–serine metabolism (5). Oral administration of D-serine reduced F4/80+ cells in the kidneys and protected from tubular injury after IR. However, antibiotic pre-treatment of WT mice, performed to deplete gut microbiota, protected from IR induced kidney injury (6). Antibiotic treated
mice also had reduced F4/80 macrophage population and chemokine receptors CX3CR1 and CCR2 in the F4/80+ renal resident macrophages. Additionally, mRNA levels of TNF-α, IL-6, MCP-1, and MIP-2α were significantly reduced in antibiotic-treated mice (6). The reason for the discrepancy between worse AKI outcomes in GF and improved AKI outcomes in antibiotic treated mice is unclear. It is possible that antibiotics selectively depleted deleterious gut microbiota with subsequent enrichment of protective gut microbiota, while there was altered maturation of kidney immune cells and function in GF mice.

**Role of gut microbiota in immune system development and function**

Gut microbiota has also been found to be instrumental in the development, induction and function of T cells, with dysbiosis leading to imbalances in T cell subpopulations. These dysregulations in gut microbiota-immune system interactions result in the development of complex immune mediated diseases including inflammatory bowel disease (IBD), rheumatoid arthritis (RA), type 1 and 2 diabetic mellitus, asthma and cancer. In addition to established immune mediated diseases, dysregulated gut microbiota-immune cell interactions influence the outcome of acute tissue injury in non-renal organs such as during models of traumatic and ischemic brain injury, myocardial infarction and acute liver injury. One study demonstrated that colonization of GF mice reduced stroke volume and post stroke neuroinflammation by priming immune responses (7). They found that lymphocyte priming, particularly of T cells by gut microbiota, was the key mechanism that affected stroke outcome. They also observed an increase in CD4, Treg and Th17 cells in the post-stroke intestines and an overall
increased T cell population in ischemic brain of colonized mice. In an experimental cardiac study, depletion of gut microbiota using antibiotics prior to injury resulted in significant mortality in mice after inducing MI (8). This study found significant reduction in myeloid cells and neutrophils in the hearts of antibiotic treated mice before and after MI, that was restored after microbiota reconstitution. Additionally, CD4+Foxp3− T cells, regulatory T cells, and B cells were absent after antibiotic treatment in the hearts. In a study on experimental acute liver injury, there was enrichment of Lactobacillus that activated IL-22 production by intestinal innate lymphoid cells, which subsequently promoted the IL-10, and TGFβ production by regulatory dendritic cells (9).

**Mechanism of gut microbiota-immune system interactions**

The mechanism by which gut microbiota interacts with kidney immune cells is likely complex and unclear but short chain fatty acids (SCFAs) appear to play important roles (10). SCFAs such as acetate, propionate and butyrate are the fermentation byproducts of dietary fibers by gut microbiota. SCFA ligation and activation of various G protein coupled receptors (GPCR) such as GPR109a, free fatty acid receptor (FFAR)2, FFAR3 and olfactory receptor (Olfr)78 are important mechanisms by which they modulate immune cell function. SCFAs can further modulate the activity of histone acetyltransferase and deacetylase and the hypoxia-inducible factor. Exogenous administration of SCFAs was found to improve kidney function during experimental AKI and contrast-induced nephropathy (10). It is likely that dysregulation in gut microbiota and microbiome-associated metabolites affect T cell functions as well as antibody mediated humoral immunity. Acetate treatment ameliorated sepsis-induced AKI by
inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase signaling in T cells suggesting that SCFAs act through immune cell regulation (11). Furthermore, \textit{in vitro} SCFA treatment was found to modulate the inflammatory process by decreasing dendritic cell maturation and inhibiting CD4 and CD8 T cell proliferation and could be a potential mechanism through which gut microbiota interact and modulate T cell functions in vivo (10). Recent data demonstrated that diet can induce posttranslational modifications to the microbial proteome that in turn affects microbial metabolite production and ultimately kidney function (12). Lack of microbes in GF mice has also been shown to impact Treg cell differentiation due to absence of SCFAs production (13).

**Conclusions**

The gut microbiota is an exciting frontier in medicine. The metagenome of the microbiota can be changed through dietary modifications as well as administration of pre, pro and post-biotics, providing unique opportunities to develop novel therapeutics for AKI treatment. Furthermore, potential therapeutic effects of AKI-specific microbiota on systemic immune dysfunction or distant organ damage in AKI remain to be explored. Although promising, diet and SCFA based AKI therapy should be investigated more carefully given evidence for potential deleterious effects such as immune cell activation, T cell-mediated ureteritis and hydronephrosis (14). Diet-based posttranslational modifications of gut microbiota proteome and metabolites should be further explored to understand effects on AKI pathophysiology (12). Since reduced diversity of gut
microbiota occurs in kidney transplantation recipients, elucidating the effect of gut microbiota on AKI could also have significant implications for allografts (15).

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**Author Contributions:** S Noel: Formal analysis; Methodology; Writing - original draft; Writing - review and editing

F Mohammad: Formal analysis; Writing - review and editing

J White: Formal analysis; Writing - review and editing

K Lee: Writing - original draft; Writing - review and editing

S Gharaie: Writing - original draft; Writing - review and editing

H Rabb: Conceptualization; Funding acquisition; Project administration; Supervision; Writing - original draft; Writing - review and editing
References


Figure legends

Figure 1. Renal ischemia reperfusion injury and cisplatin treatment change gut microbial populations. Acute kidney injury (AKI) was induced in male 8-10 week old C57BL/6 mice under an approved animal protocol by 30 min bilateral IR or 30 mg/kg cisplatin (CP) injection, then gut microbiota studied at baseline (D0) and 72h (D3) post AKI using 16s sequencing. (A) IR and CP affected relative abundance of bacterial species belonging to phylum Actinobacteria, Bacteroidetes, Firmicutes, Tenericutes and Verrucomicrobia. (B) The vertical dot plots represent differential abundance testing between Sham and CP or Sham and IRI (x axis = fold change, size = base mean) showing distinct alterations in microbial populations at family level. The genus identified on the y-axis are those that impacted by AKI, using negative binomial testing. There were no significant OTUs with species level information. (C&D) Dimensional analysis by Brey NMDS and alpha diversity analysis using Shannon index suggests the microbiome differentiates itself over time depending on treatment. (E) Percent change in Erysipelotrichaceae incertae sedis and Lactobacillus population after IRI and CP–induced AKI.

Figure 2: An overview of gut microbiota - immune cell interactions in the kidney.
Gut microbiota produces SCFAs such as acetate, propionate and butyrate that interact with multiple G protein receptors on kidney epithelial cells. Similar interactions likely are involved in immune cells that modulates their number, immune function and metabolism in the kidney. Normal gut microbiota promote anti-inflammatory milieu by increasing Th2, Treg and M2 macrophage populations that protect kidneys from AKI. However, AKI
induced dysbiosis and translocation of bacterial products across leaky intestine promote proinflammatory immune environment such as increased Th1 cells, M1 macrophages and activated DCs. Activated DCs secrete proinflammatory cytokines such as IL-12, IL-6 and skew the differentiation of naive CD4 T cells and maturation of B cells. Th17-inducing bacteria may promote Th17 immunity via IL-17A/IL-17F induction, which may involve signaling mediated by the TLR ligands. Additionally, IgA produced by plasma cells residing in the gut epithelium modulates response to colonization by specific commensal bacteria. SCFAs also regulate cytokine expression in T cells and generation of Tregs through HDAC inhibition. Therapeutic and supplemental use of pre, pro and post-biotics could be helpful in normalizing bacterial composition in the gut and protect kidneys from AKI.
Prebiotics

Probiotics

Gut microbiota

Dietary fiber

Th2 cell

Treg cell

M2 macrophage

SCFAs

NKT cell

DC

AKI injury

AKI repair

Figure 2