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REVIEW

The consequences of altered microbiota in immune-related chronic kidney disease

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ABSTRACT

The normal gut microbiome modulates host enterocyte metabolism and shapes local and systemic immunity. Accumulation of urea and other waste products in chronic kidney disease induces gut dysbiosis and intestinal wall inflammation (leaky gut). There are decreased numbers of bacteria that generate short-chain fatty acids, which are an important nutrient source for host enterocytes and also contribute to regulation of the host immune system. Anaerobic proteolytic bacteria that express urease, uricase and indole and *p*-cresol enzymes, such as Enterobacteria and Enterococci, are increased. Microbial-derived uremic toxins such as indoxyl sulfate and trimethylamine *N*-oxide contribute to the pathophysiology of immune-related kidney diseases such as diabetic nephropathy, lupus nephritis and immunoglobulin A (IgA) nephropathy. Animal and clinical studies suggest potential benefits of dietary and probiotic interventions in slowing the progression of immune-related kidney diseases.

Keywords: chronic kidney disease, diabetic nephropathy, gut microbiome, IgA nephropathy, lupus nephritis

THE GUT MICROBIOME IN HEALTH

The healthy adult gastrointestinal tract harbors ∼100 trillion microbes including 2000–4000 species, both aerobic and anaerobic. This is 10-fold greater than the number of cells in the human body [1, 2]. The genome of the gut microbiome encompasses 3.3 million genes, which is at least 150-fold larger than the human genome [3]. Bacteria are the most abundant (>50 phyla); archaea and eucarya are also present, and microbial density is the highest in the colon [4]. The anaerobic phyla Bacteroidetes (genus-level examples include *Bacteroides*, *Prevotella*) and Firmicutes (*Clostridium*, *Eubacterium*, *Roseburia* and *Ruminococcus*) contribute >90% of bacterial species [3]. Although the diversity of the human gut microbiota is influenced by gender, ethnicity, immune status, nationality, age, diet, geographic location, alcohol and drug consumption, and smoking [5, 6], >50% of healthy individuals share the same 75 bacterial species [3]. Bacterial abundance and diversity increase from the stomach (10^2–10^4 cells/mL) to the colon (>10^{12} cells/mL) as oxygen tension declines [7].

Gut microbes evolved symbiotically and confer important benefits to the host. The microbiota is involved in gut motility, modulates energy harvesting and absorption of complex carbohydrates, and is a source of micronutrients including short-chain fatty acids (SCFAs), vitamins such as B vitamins and vitamin K6, and amino acids such as lysine and threonine [8, 9]. SCFAs include acetate, butyrate, propionate and D-lactate, and are derived from fermentation of starch in the colon by Bacteroides; host enterocytes derive 60–70% of their energy from SCFA oxidation [10]. SCFAs from gut microbiota metabolism are present in hepatic, portal and peripheral blood, and influence lipid, glucose and cholesterol metabolism in various tissues [10]. SCFAs bind and activate G-protein coupled receptors free fatty acid receptor 2 (FFAR2, also called GPR 41) and free fatty acid receptor 3 (FFAR3, also called GPR43) expressed on immune cells, endocrine cells, enterocytes, adipose tissue and the autonomic nervous system, and modulate host energy homeostasis [2].

The gut microbiota offers direct protection against pathogens via production of antibiotics and bacteriocins. The gut mucosal barrier, consisting of a mucus layer, defensins and lectins, shields the epithelium from direct contact with microbiota [11]. Nonetheless, intestinal microbes modulate development of local as well as systemic immunity (Figure 1), shaping the composition of T cell and natural killer cells subsets as discussed below.

GUT MICROBIOTA SHAPE THE HOST IMMUNE SYSTEM

The normal microbial flora colonizes the intestinal tract after birth and modulates antigen-responsiveness of the lymphoid tissues [12]. Evidence from germ-free mice indicates that the gut microbiota is involved in the development of gut-associated lymphoid tissues including Peyer's patches and mesenteric lymph nodes, antibody production [immunoglobulin A (IgA)

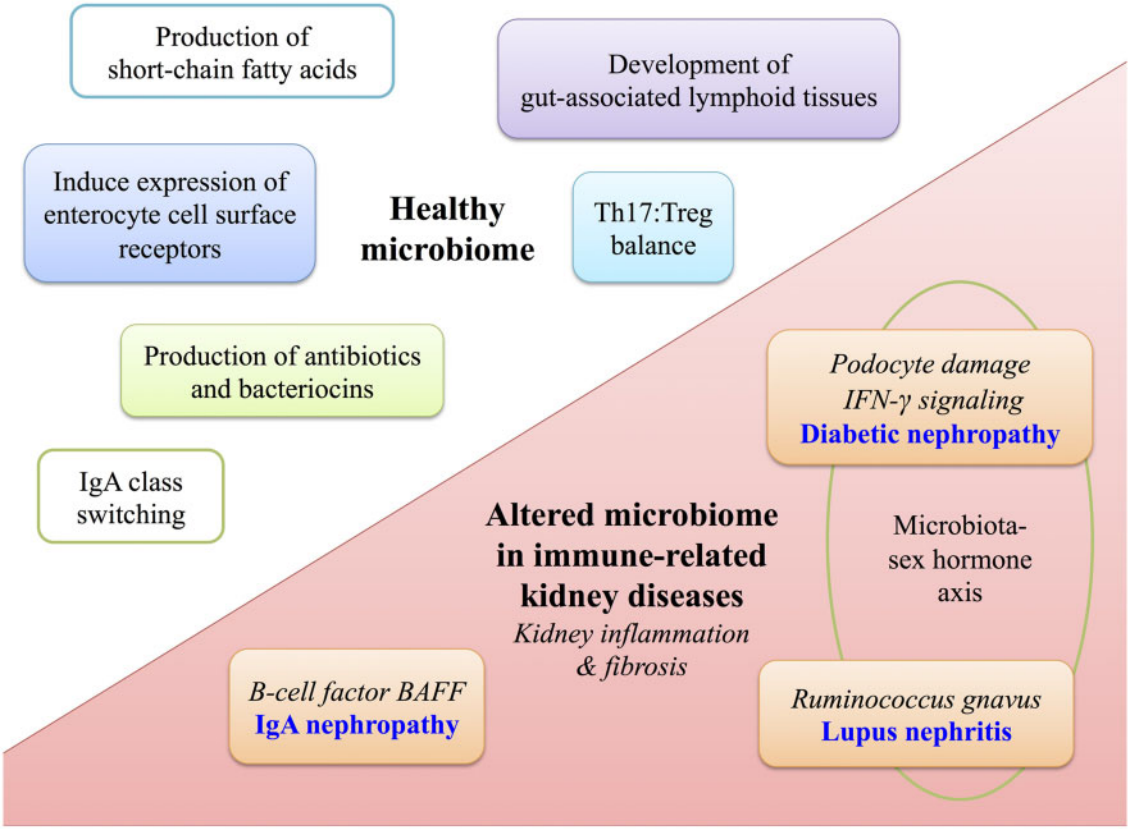

FIGURE 1: Role of the gut microbiome in health and in autoimmune kidney diseases.
Upper left: the healthy gut microbiome shapes local and systemic immunity including production of antibiotics and bacteriocins, induction of surface pattern recognition receptors on host intestinal cells and production of SCFAs. SCFAs provide a nutrient source to host intestinal cells and also modulate immune function via induction of natural killer cells and expansion of Tregs. The microbiota also modulates development of gut-associated lymphoid tissues (Peyer's patches, mesenteric lymph nodes), shapes the balance between cytotoxic Th17 and Treg cells and induces IgA class switching. Lower right: the microbiome is altered in CKD and bacterial-derived uremic toxins translocate across the leaky gut and induce systemic effects including kidney inflammation and fibrosis. In diabetic nephropathy, the toxin phenyl sulfate induces podocyte damage and proteinuria. There is a microbiota–sex hormone axis whereby transfer of microbiota from male to female mice increases IFN-γ signaling and reduced incidence of diabetes. In lupus nephritis, higher gut abundance of *Ruminococcus gnavus* strain 2 is associated with more severe lupus nephritis kidney pathology. A microbiota–sex hormone axis is also observed, whereby female mice have more severe disease. Mouse studies indicate that overexpression of BAFF, a B-cell factor crucial for IgA synthesis, develop a commensal flora-dependent IgA nephropathy.

class switching] and expression of surface pattern recognition receptors on intestinal epithelial cells [13].

Two T-cell subsets, regulatory T cells (Tregs) and T helper 17 (Th17) cells are enriched in the intestinal lamina propria and play critical roles in host defense and homeostasis. Th17 cells produce interleukin (IL)-17 and IL-22, two cytokines that stimulate the intestinal epithelium to produce anti-microbial peptides; Th17 cells are also critical for recruitment of neutrophils to eliminate bacteria that have traversed the epithelium [12]. If the latter is left unchecked, then there can be excessive gut wall inflammation with tissue destruction. Treg cells in turn are critical in providing immunologic self-tolerance countering Th17 effects, to avoid excessive inflammation; the gut microbiota shapes this balance between effector and Tregs [12]. SCFAs produced by Bacteroides also contribute to immune system activation through (i) neutrophil chemotaxis, (ii) induction of natural killer cells and (iii) differentiation and proliferation of Tregs (butyrate is the most potent SCFA in Treg expansion) [14, 15].

GUT DYSBIOSIS IN CHRONIC KIDNEY DISEASE

Disruptions in the gut microbiome have been implicated in progression of numerous conditions including chronic kidney disease (CKD), inflammatory bowel disease, metabolic disorders, cardiovascular diseases, cancer and allergic disorders [1, 16]. Simenhoff *et al.* [17] performed endoscopy studies in the 1970s and were the first to demonstrate markedly altered gut flora in CKD patients. They noted that antibiotic treatment altered the microbiome, decreased serum levels of amine toxins and patients reported improved mentation [18]. The duodenum and jejunum, which are only lightly colonized in the healthy individual, become intensely colonized by aerobic and anaerobic bacteria in CKD patients [17].

As further evidence of gut dysbiosis, gas chromatography studies have shown significantly altered exhaled breath gases in CKD rats [19] and end-stage kidney disease (ESKD) patients [20] compared with healthy controls. It has been proposed that

influx of urea and other retained toxins in CKD, from the bloodstream into the gut lumen, selects for microbes that express urease, uricase and indole and *p*-cresol-forming enzymes [21, 22]. These changes in the gut flora are exacerbated by the CKD diet, which by virtue of being low in potassium and phosphorus equates to a diet low in fermentable plant fiber and poor in symbiont-rich cheese/yogurt [16]. The fiber-poor CKD diet also impacts microbial production of beneficial SCFAs; transit of plant-derived resistant starches to the colon is necessary for fermentation by Bacteroides to release hydrogen, carbon dioxide, alcohol and SCFAs [7]. Indeed, fecal analysis has demonstrated that dialysis patients show decreased numbers of bacteria that are able to produce the SCFA butyrate [22].

In an earlier study, our group isolated stool microbial DNA from ESKD patients on hemodialysis and a group of age-, sex- and ethnicity-matched healthy individuals. Via a phylogenic microarray technique, we demonstrated highly significant differences in the abundance of >200 bacterial operational taxonomic units (OTUs) belonging to 23 bacterial families between the ESKD and the healthy control groups; of note, there were lower numbers of Lactobacillaceae and Prevotellaceae families (both are considered normal colonic microbiota) and 100 times higher numbers of Enterobacteria and Enterococci anaerobic species (which are normally present in lower proportion) [21]. In order to isolate the effects of uremia from those of comorbid conditions, medications, diet and other inter-individual variations, the stool microbiome was studied in rats 8 weeks after 5/6 nephrectomy or sham operation. The CKD rats showed significant differences in the abundance of 175 bacterial OTUs, thus confirming the effect of CKD on gut dysbiosis [21]. In a subsequent study, stool microbial genomic analyses confirmed that hemodialysis patients demonstrated expansion of bacteria that express urease, uricase and *p*-cresyl- and indole-forming enzymes concurrent with depletion of the bacteria that produce SCFAs [22]. Among the 19 microbial families that were dominant in ESKD patients, 12 possessed urease (Alteromonadaceae, Cellulomonadaceae, Clostridiaceae, Dermabacteraceae, Enterobacteriaceae, Halomonadaceae, Methylococcaceae, Micrococcaceae, Moraxellaceae, Polyangiaceae, Pseudomonadaceae and Xanthomonadaceae), five possessed uricase (Cellulo-monadaceae, Dermabacteraceaea, Micrococcaceae, Polyangiaceae and Xanthomonadaceae) and three possessed indole and *p*-cresol-forming enzymes (Clostridiaceae, Enterobacteriaceae and Verrucomicrobiaceae). Lactobacillaceae and Prevotellaceae, two species that generate the SCFA butyrate, were among the four microbial families that were depleted in ESKD patients [22].

Gut dysbiosis has similarly been reported in pediatric ESKD patients. Crespo-Salgado *et al.* examined the gut microbiome in pediatric patients undergoing hemodialysis or peritoneal dialysis; increased Bacteroidetes with decreased Proteobacteria was noted in pediatric hemodialysis patients, while increased Enterobacteriaceae and decreased Firmicutes and Actinobacteria was noted in pediatric peritoneal dialysis patients [23]. Increased numbers of glucose-fermentable bacteria Enterobacteriaceae in the setting of peritoneal dialysis was attributed to intestinal absorption of glucose from intraperitoneal dialysate [23]. It is important to note that despite the accumulating data regarding gut dysbiosis in animals and humans with CKD, it remains unknown to what degree these differences in the microbial subpopulations change microbial function and contribute to disease states.

In tandem with gut dysbiosis, there is widespread intestinal inflammation and increased gut permeability in CKD, with systemic consequences. Vaziri *et al.* described chronic inflammation throughout the gastrointestinal tract—extending from esophagus to large bowel—in chronic hemodialysis patients on autopsy studies performed in the 1980s [24]. In the early 1990s, studies utilizing polyethylene glycols of various molecular weights demonstrated increased permeability of the intestinal wall in CKD patients and rats [16]. To date, two mechanisms have been described that led to tight junction breakdown [16, 25]. Urea diffuses from the blood into the gut lumen and is metabolized by gut bacterial urease to ammonia. Ammonia is subsequently hydrolyzed into caustic ammonium hydroxide, which erodes the epithelial barrier and stimulates influx of inflammatory leukocytes. This triggers the second mechanism whereby local cytokine production induces retraction and endocytosis of transcellular tight junction proteins including claudins and occludin. Animal studies have confirmed that CKD is associated with depletion of gut epithelial tight junction proteins [26, 27].

This leaky gut barrier facilitates systemic translocation of gut bacterial DNA and products of bacterial protein catabolism; the latter generate uremic toxins [e.g. indoxyl sulfate, *p*-cresyl sulfate, indole-3 acetic acid, trimethylamine *N*-oxide (TMAO) and phenylacetylglutamine], which are detectable in the blood of CKD and ESKD patients [1]. Tryptophan is metabolized into indole by intestinal bacteria and is subsequently sulfated in the liver to form indoxyl sulfate. *p*-cresol/*p*-cresyl sulfate is derived from phenylalanine and tyrosine; *p*-cresol is conjugated by intestinal microbes to *p*-cresyl sulfate (the main circulating toxin) and *p*-cresyl glucoronidate (which is metabolized to *p*-cresyl glucuronide in the liver). TMAO is derived from bacterial metabolism of quaternary amines, such as phosphatidylcholine or L-carnitine which generates trimethylamine, with subsequent conversion to TMAO by flavin monooxygenase enzymes in the liver.

A definitive study by Aronov *et al.* [28] confirmed the colonic origin of microbial-derived uremic toxins (including *p*-cresyl sulfate and indoxyl sulfate) by mass spectroscopy analysis of plasma solutes from ESKD patients who had undergone total colectomy. Compared with ESKD and control subjects who had intact colons, the colectomized ESKD individuals had significantly lower or absent plasma levels of >30 solutes [28]. Nearly all of these compounds were significantly lower in healthy individuals, suggesting that they represented uremic solutes. These findings provide irrefutable evidence for colonic microbes being the source of numerous uremic solutes—many of which have yet to be identified.

GUT DYSBIOSIS AND IMMUNE-RELATED KIDNEY DISEASES

Gut-derived uremic toxins have been shown to promote kidney inflammation and fibrosis in animal models including 5/6 nephrectomized rats and ApoE-knockout mice [1]. Below we

Table 1. Changes in the gut microbiota in CKD and in immune-related kidney diseases

Chronic disease state	Increased genera	Decreased genera
CKD	Adult dialysis patients: Enterobacteriaceae, Clostridia, Bacteroidia and Pseudomonadaceae Pediatric dialysis patients: Enterobacteriaceae, Bacteroidia	Adult dialysis patients: Bifidobacteriaceae, Lactobacillaceae and Prevotellaceae Pediatric dialysis patients: Proteobacteria, Firmicutes and Actinobacteria
Diabetic nephropathy	Type 1 diabetes mellitus: Bacteroides, Blautia, Rikenellaceae, *Ruminococcus*, *Streptococcus*, *Clostridium perfringens*, *Veillonella* and *Fusobacteria* Type 2 diabetes mellitus: *Escherichia*, *Prevotella*, *Clostridium bolteae*, *Clostridium symbiosum*, *Clostridium ramosum*, *Clostridium hathewayi*, Betaproteobacteria and Desulfovibrio	Type 1 diabetes mellitus: Lactobacillaceae, *Bifidobacterium*, *Prevotella*, *Staphylococcus*, Lachnospiraceae, Veillonellaceae, *Coprococcus eutactus, Dialister invisus*, *Roseburia faecis*, *Faecalibacterium prausnitzii*, *Clostridium clostridioforme*, *Blautia coccoides*, *Pseudobutyrivibrio* and *Akkermansia muciniphila* Type 2 diabetes mellitus: *Bifidobacterium*, Lactobacillaceae, *Eubacterium rectale*, Faecalibacterium, *Roseburia intestinalis*, Clostridiales, *Akkermansia muciniphila* (Verrucomicrobiaceae) and *Streptococcus*
Lupus nephritis	Lachnospiraceae, Lactobacillaceae, Bacteroides and RG	Firmicutes
IgA nephropathy	Sutterellaceae, Enterobacteriaceae and Firmicutes (latter includes genera/species of Ruminococcaceae, Lachnospiraceae, Eubacteriaceae and Streptococcaeae)	*Clostridium*, *Lactobacillus*, *Enterococcus* and *Bifidobacterium*

summarize evidence known to date about gut microbiome interactions with immune-related kidney diseases such as diabetic nephropathy, lupus nephritis and IgA nephropathy (Table 1 and Figure 1). Other end-organ dysfunction (cardiovascular, hematologic, neurohormonal, etc.) induced by systemic effects of gut-derived uremic toxins from the altered microbiome has been discussed in previous reviews [1, 2, 16, 25].

DIABETIC NEPHROPATHY

In a study of 14 patients with biopsy-proven diabetic nephropathy, Tao *et al.* reported significant differences in expression of the *Escherichia-Shigella* and *Prevotella* genera compared with diabetic individuals who did not have kidney disease [29]. Dietary habits were not a determining factor, as household contacts of the diabetic patients had similar microbiota composition to non-household healthy controls. The investigators proposed that increased levels of *Eschericha-Shigella* contribute to the pathophysiology of diabetes by promoting breakdown of the gut epithelial barrier and by producing ethanol, which leads to disordered fatty acid metabolism [29]. Alterations in *Lactobacillus* and *Bifidobacterium* genera are common trend seen across proteinuric kidney diseases (Table 1) [30]. As further evidence of the important role of the gut microbiota in kidney disease, frequent use of antibiotics (which disrupts the balance of normal intestinal flora) has been shown to correlate with severity of diabetic nephropathy in patients with Type 1 diabetes [31].

Kikuchi *et al.* demonstrated that phenyl sulfate, derived from gut microbial metabolism of tyrosine, contributes to podocyte damage and albuminuria in animal CKD models [32]. They generated transgenic rats overexpressing the organic anion transporting polypeptide transporter SLCO4C1 in the proximal tubule to amplify renal excretion of uremic toxins; transgenic rats with streptozotocin-induced diabetes had less proteinuria than wild-type diabetic rats. Further investigations were done in diabetic mouse models (db/db and KKAy mice on high-fat diet), whereby oral administration of phenyl sulfate induced albuminuria and podocyte damage. Inhibition of tyrosine phenol-lyase, a bacterial enzyme responsible for phenol synthesis from dietary tyrosine before it is metabolized into phenyl sulfate in the liver, reduced albuminuria in diabetic mice. Kikuchi *et al.* also reported findings from the urinary biomarker for continuous and rapid progression of diabetic nephropathy patient cohort whereby baseline blood phenyl sulfate levels predict 2-year progression of albuminuria in patients with baseline microalbuminuria [32]. Similar to the autoimmunity in systemic lupus (discussed below), there appears to be a microbiota–sex hormone axis in Type 1 diabetes; transfer of microbiota from male to female mice was shown to raise testosterone and reduce diabetes incidence in female animals, partly due to enhanced Type I interferon (IFN)-γ signaling [33]. *p*-cresyl sulfate and TMAO via inducing accelerated aging pathways also likely contribute to the microvascular complications of diabetes including nephropathy [34]. Diabetes is independently associated with higher free *p*-cresol serum concentrations after adjustment for kidney function [35], suggesting that this gut-derived microbial toxin may be a shared pathologic factor in both diabetes and CKD.

LUPUS NEPHRITIS

Systemic lupus erythematosus (SLE) is a complex autoimmune disease associated with excessive signaling of RNA-sensing Toll-like receptor 7 and Type I IFNs. Investigations of gut microbiota using animal SLE models have generated varying results. In MRL/lpr lupus-prone mice, severity of lupus disease indices correlated positively with increased levels of Lachnospiraceae in gut microflora but was inversely correlated with Lactobacillaceae (which was decreased in the gut microbiome) [36]. In contrast, the NZB/W F1 mouse SLE model demonstrates increased abundance of gut Lactobacillaceae and this increase was reversed by dexamethasone [37]. Fecal samples from SLE patients have demonstrated increased Lactobacillus species, and decreased amount of Firmicutes relative to Bacteroidetes [38–41]. Recently, Azzouz *et al.* reported that the abundance of gut *Ruminococcus gnavus* (RG) was

associated with lupus disease activity score (Systemic Lupus Erythematosus Disease Activity Index score) and correlated inversely with C3 and C4 complement levels [42]. Furthermore, highest levels of serum anti-RG antibodies against the RG-2 strain (not RG-1) were detected in those with proliferative lupus nephritis (including Classes III and IV). The findings were validated in two small independent SLE cohorts that include both men and women of diverse ethnic background [42].

The exact molecular pathways linking gut microbiota to SLE and lupus nephritis are not fully elucidated. Emerging data from animal and human studies support several mechanisms including translocation of microbial antigens from the leaky gut, molecular mimicry and the microbiota–sex hormone axis. First, SLE patients exhibit limited gut microbiota diversity and gut-derived pathobionts including *Enterococcus gallinarum* are detectable in mesenteric lymph nodes and the liver [43]. Animal studies show that gut-derived *E. gallinarum* reaches the liver and induces development of lupus autoantibodies, partly via activating the aryl hydrocarbon receptor-CYP1A1 pathway triggering Th17 cell activation and anti-dsDNA antibody production [43]. Second, molecular mimicry of human autoantigens by bacterial orthologs such as 60 kDa Ro protein (Ro60) triggers cross-reactive T- and B-cell autoimmune responses [40]. Third, the microbiota–sex hormone axis has been implicated similar to that observed in Type 1 diabetes pathophysiology. In mouse SLE models, gut microbiota differ between female and male lupus-prone mice and the female mice have more severe disease [36]; castration of male mice reversed this difference indicating an androgen-dependent pathway [33].

IgA NEPHROPATHY

IgA nephropathy patients show increased stool Sutterellaceae, Enterobacteriaceae and Firmicutes, while levels of *Clostridium*, *Enterococcus*, *Bifidobacterium* and *Lactobacillus* are decreased, compared with healthy controls [44]. IgA nephropathy is characterized by circulating immune complexes containing aberrantly glycosylated IgA1, and prominent mesangial IgA deposition in the kidneys. Recent data suggest that gut-associated lymphoid tissue plays a major role in the development of IgA nephropathy. Genome-wide association studies showed that most loci associated with the risk of IgA nephropathy are also associated with inflammatory bowel disease, suggesting that disruption of the intestinal barrier contributes to disease pathogenesis [45]. This is further supported by data from experimental models. For example, mice overexpressing BAFF, a B-cell factor crucial for IgA synthesis, develop a commensal flora-dependent IgA nephropathy [46].

PRE- AND PROBIOTICS IN CKD AND AUTOIMMUNE DISEASES

Prebiotics (nondigestible food ingredients that can stimulate growth and/or activity of beneficial gut bacteria) and probiotics (living organisms ingested via food or supplements that can improve the health of the host) are being actively studied as approaches to dampen chronic inflammation and improve quality of life in CKD patients (Table 2). Our group previously reported that feeding uremic rats with high amylose-resistant starch (a prebiotic) improved creatinine clearance and reduced kidney inflammation and fibrosis [48]. Resistant starches transit to the colon undigested and are metabolized by bacteria to SCFAs, which, as discussed above, are important nutrients to enterocytes. A recent randomized controlled trial of 46 hemodialysis patients in Iran noted that a diet enriched with amylose (HAM-RS2 biscuits) for 8 weeks significantly reduced serum tumor necrosis factor (TNF)-α, IL-6 and urea with patients reporting less constipation [49]. Total serum antioxidant activity and hs-CRP levels were unchanged compared with placebo (wheat-flour biscuits). Other small trials in ESKD patients utilizing oligofructose–inulin or resistant starch supplementation have demonstrated significant reduction in circulating levels of gut-derived uremic toxins such as indoxyl sulfate and *p*-cresyl sulfate [50, 51].

Probiotics have been tested in CKD with the goal of producing a less pathogenic microflora and thus reduce generation of uremic toxins. However, a randomized controlled trial with the Renadyl formulation of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium longum* in 22 patients with CKD Stages 3 and 4 failed to reduce plasma concentrations of uremic toxins and did not improve quality of life parameters [52]. The lack of benefit with probiotics may be explained by persistent uremia-induced changes in the gut biochemical environment combined with dietary restrictions and medications, which create an unfavorable milieu for the symbiotic microbiota. Thus, attempts to restore the desired microbiome without simultaneously improving the gut's biochemical milieu can be futile. To address this deficit, the Synbiotics Easing Renal Failure by Improving Gut Microbiology (SYNERGY) trial examined the combination of pro- and prebiotic therapy (symbiotic formulation) >6 weeks in predialysis CKD patients and noted decreased serum *p*-cresyl sulfate and improvement in gut microbiome diversity; however, systemic markers of inflammation and proteinuria were not changed [54]. Studies of longer duration are needed to define clinical impact of pre- and probiotics in the CKD population. We also caution that choice of probiotic microbe is important; inclusion of bacteria that express urease with the intention to metabolize gut urea may be misguided since the downstream products ammonia and ammonium hydroxide can promote intestinal wall inflammation, as discussed above [16, 25].

Probiotics have been studied in CKD-related autoimmune diseases including Type 1 diabetes mellitus and SLE. In nonobese diabetic mice, oral administration of probiotic enriched in Lactobacillaceae prevented development of overt Type 1 diabetes, decreased IL-1β and increased CD103+ tolerogenic dendritic cell differentiation in the gut [55]. The TEDDY study evaluated the effects of early probiotic supplementation in children with genetic risk factors for Type 1 diabetes [53]. This multicenter prospective study involved 7473 children aged 4–10 years. Early probiotic exposure within 27 days of life correlated with decreased risk of islet cell autoimmunity. In a randomized controlled trial of 70 nonobese Iranian adults with

Table 2. Studies of prebiotics and probiotics in immune-related kidney diseases

Chronic disease state	Intervention	*n* per group	Outcomes
Animal studies			
Rat adenine CKD model [46] *Prebiotic study*	High amylose maize resistant starch (HI-MAIZE 260)	CTL $n = 6$ CKD $n = 9$ CKD-HI-MAIZE $n = 9$	Improved expression of epithelal tight junction proteins in the colon; reduced kidney inflammation and fibrosis; improved creatinine clearance
Mouse MRL/lpr lupus nephritis model [47] *Probiotics study*	Mix of five Lactobacillus strains	PBS control $n = 7$ Probiotics $n = 7$	Improved gut epithelial barrier, decreased IgG2a, increased IL-10 and improved Treg–Th17 balance in the kidneys at 7 weeks. Benefits seen only in female mice and castrated male mice, suggesting modulation of the microbiota by sex hormones
Human studies			
Chronic hemodialysis patients in Iran [48] *Prebiotic study*	Amylose resistant starch (HAM-RS2)	Wheat-flour control $n = 23$ HAM-RS2 $n = 23$	Decreased serum levels of TNF-α, IL-6 and malondialdehyde at 4 weeks Decreased constipation No significant difference in serum IL-1β, hs-CRP and total antioxidant activity
Chronic hemodialysis patients in Belgium [49] *Prebiotic study*	Oligofructose-enriched inulin (ORAFTI®Synergy 1, Tienen, Belgium)	$n = 22$ All patients subjected to escalating dose regimen	Serum *p*-cresyl sulfate significantly reduced at 4 weeksNo change in indoxyl sulfate
Chronic hemodialysis patients in USA [50] *Prebiotic study*	High-amylose corn starch (Hi-maize 260)	CTL $n = 20$ Hi-maize $n = 20$	Decreased serum levels of indoxyl sulfate at 6 weeksNo change in *p*-cresol sulfate
Chronic hemodialysis patients in USA [51] *Probiotics study*	Renadyl formulation: 30 billion CFU of *S. thermophilus* KB 19, *L. acidophilus* KB 27, and *B. longum* KB 31	$n = 22$ Placebo-controlled crossover study	No significant change in uremic toxin levels or quality of life scores at 6 months
Predialysis CKD Stages 4–5 patients in Australia [52] *Symbiotic study*	Prebiotics: combination of high-molecular weight inulin (inulin high performance), fructo-oligosaccharides and galacto-oligosaccharides Probiotics: nine different strains across the *Lactobacillus*, *Bifidobacteria* and *Streptococcus* genera	$n = 31$ Placebo-controlled crossover study	Serum *p*-cresyl sulfate decreased at 6 weeks. Increased stool Bifidobacterium and decreased Ruminococcaceae No change in indoxyl sulfate, endotoxin level, serum inflammatory biomarkers (IL-1β, IL-6, IL-10 and TNF-α), oxidative stress biomarkers (F_2-isoprostanes and glutathione peroxidase), urinary Kim-1 or proteinuria Increase in albuminuria of 38 mg/24 h
Non-obese patients with Type 2 diabetes mellitus in Iran [53] *Symbiotic study*	Prebiotic: Fructo-oligosaccharide Probiotics: *Lactobacillus*, *Bifidobacterium* and *Streptococcus thermophiles*	Placebo $n = 35$ Symbiotic therapy $n = 35$	Significant decrease in HbA1c and albuminuria at 9 weeksNo change in urea, creatinine or lipid profile

Type 2 diabetes, symbiotic therapy prevented increase in microalbuminuria and Hgb A1c, while there was no effect on markers of kidney function [47].

Studies targeting gut microbiome dysbiosis in SLE have been restricted to animal models. In MRL/lpr lupus-prone mice, *Lactobacillus* probiotic treatment reduced proteinuria and kidney disease activity (assessed on biopsy samples) and prolonged survival [56]. *Lactobacillus* supplementation restored IL-10 production and decreased circulating IgG2a, an antibody isoform that tends to deposit in the kidneys of MRL/lpr mice. Flow cytometry studies of kidney tissue demonstrated that *Lactobacillus* skewed the Treg–Th17 balance toward a less inflammatory Treg phenotype. The findings were sex-dependent; benefits of *Lactobacillus* supplementation were observed in female and castrated male mice, but not in noncastrated male mice [56], consistent with a microbiota–sex hormone axis (discussed above). To date, clinical trials using probiotics as adjuvant therapy in SLE patients are lacking.

MEDICATION EFFECTS ON THE GUT MICROBIOTA

Disease-specific and immunomodulatory medications can impact the gut microbiome. Metformin, an established oral hypoglycemic agent, has been reported to increase beneficial gut bacteria that produce SCFAs [57]. The newer sodium-glucose cotransporter 2 (SGLT2) inhibitors were reported in animal investigations to alter the gut microbiome and decrease serum levels of indoxyl and *p*-cresyl sulfates [58, 59]; however, a randomized trial in patients with Type 2 diabetes found no change

in microbiome composition with the SGLT2 inhibitor dapagliflozin [60]. Steroids are a common treatment for lupus nephritis and IgA nephropathy; dexamethasone treatment in NZB/W F1 lupus-prone mice was shown to increase gut microbial diversity while lowering abundance of pathogenic Lactobacillaceae [37]. Conversely, animal studies examining transplant immunosuppression medications (including prednisolone, tacrolimus, sirolimus or everolimus, mycophenolate mofetil) have demonstrated significant decrease in bacterial diversity leading to a more catabolic microbial profile [61, 62]. The combination of prednisolone, tacrolimus and mycophenolate mofetil induced variable changes in the fecal microbiota; of concern, a dramatic increase in uropathogenic *Escherichia coli* strain 536 was noted [62]. In a small trial of 19 kidney transplant recipients, fecal abundance of *Faecalibacterium prausnitzii* was significantly higher in patients who required dose escalation of tacrolimus to achieve therapeutic blood levels [63]. While evidence in this area is still exploratory, the data suggest that alterations to the gut microbiome with immunomodulatory medications can in turn affect host response to therapy for autoimmune-related kidney diseases.

CONCLUSIONS

The gut microbiome is altered in CKD with lower numbers of Lactobacillaceae and Prevotellaceae families and higher concentrations of anaerobic species that express urease, uricase, and *p*-cresyl- and indole-forming enzymes. Production of SCFAs falls, depriving host enterocytes of an important nutrient source and immune-modulating signal. Microbe-derived uremic toxins such as indoxyl sulfate, *p*-cresyl sulfate and TMAO induce systemic inflammation and widespread end-organ damage including pathogenesis of immune-related CKD states such as diabetic nephropathy, IgA nephropathy and lupus nephritis. Additional studies are needed to better understand gut dysbiosis in these disease states, and to examine potential benefits of gut-targeted interventions to restore a balanced microbiome in these patients.

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AUTHORS' CONTRIBUTIONS

W.L.L. and Y.C. drafted the manuscript and N.D.V. participated in manuscript revision.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. This paper has not been published previously in whole or part.

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