Role of the Gut Microbiome in Uremia: A Potential Therapeutic Target

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Also known as the “second human genome,” the gut microbiome plays important roles in both the maintenance of health and the pathogenesis of disease. The symbiotic relationship between host and microbiome is disturbed due to the proliferation of dysbiotic bacteria in patients with chronic kidney disease (CKD). Fermentation of protein and amino acids by gut bacteria generates excess amounts of potentially toxic compounds such as ammonia, amines, thiols, phenols, and indoles, but the generation of short-chain fatty acids is reduced. Impaired intestinal barrier function in patients with CKD permits translocation of gut-derived uremic toxins into the systemic circulation, contributing to the progression of CKD, cardiovascular disease, insulin resistance, and protein-energy wasting. The field of microbiome research is still nascent, but is evolving rapidly. Establishing symbiosis to treat uremic syndrome is a novel concept, but if proved effective, it will have a significant impact on the management of patients with CKD.

INDEX WORDS: Gut microbiome; uremic toxin; microbial metabolite; metabolome; ammonia; urea; amine; thiol; phenol; indole; p-cresyl sulfate (PCS); uremic syndrome; chronic kidney disease (CKD); end-stage renal disease (ESRD); review.

BACKGROUND

Findings from the Human Microbiome Project (HMP) and the Metagenomics of the Human Intestinal Tract (Meta-HIT) project have shown that the human intestine is home to an extraordinarily complex and dynamic consortium of bacteria that play a pivotal role in human health and disease.1,2 Bacteria have co-evolved with humans, and this symbiotic relationship has expanded our capabilities beyond what is coded in our own genome.3 Genetically, we are vastly outnumbered by our own microbiome, the microbial genome. As the Nobel Laureate Joshua Lederberg has asserted, “We should think of each host and its parasites as a superorganism with the respective genomes yoked into a chimera of sorts.”4p9 The central role of the gut in human health has been long recognized, dating back to 400 BC, when Hippocrates stated, “Death sits in the bowels.”5 This review provides an overview of the bidirectional relationship between chronic kidney disease (CKD) and the gut microbiome, discusses the consequences of gut dysbiosis in the pathogenesis of systemic inflammation and uremic toxicity, and highlights the recent advances in targeting the gut microbiome for therapeutic purposes.

CASE VIGNETTE

A 65-year-old man with CKD stage G4 presented with lethargy and chronic constipation to the emergency department. Clinical examination findings were unremarkable except for generalized muscle weakness and a distended abdomen with sluggish bowel sounds. Laboratory investigation showed the following values: sodium, 138 mEq/L; potassium, 6.3 mEq/L; chloride, 115 mEq/L; bicarbonate, 16 mEq/L; anion gap, 14; serum urea nitrogen, 60 mg/dL; serum creatinine, 3.8 mg/dL (corresponding to estimated glomerular filtration rate [eGFR] of 16 mL/min/1.73 m2 using the IDMS-traceable 4-variable MDRD Study equation); glucose, 100 mg/dL; calcium, 8.1 mg/dL; phosphate, 7.1 mg/dL; albumin, 3.6 g/dL; white blood cell count, 8.1 × 109/L; and hemoglobin, 10.1 g/dL. Computed tomography of the brain was normal except for mild cortical atrophy. Computed tomography of the abdomen showed abundant fecal matter in a dilated rectum and sigmoid colon. After disimpaction with enemas and laxatives, the patient felt better. He was discharged with the recommendation to take laxatives on a regular basis. The patient was seen in the outpatient clinic 2 months later. He appeared energetic and said that he was taking a prebiotic (p-inulin) and continuing the laxative when needed. Repeat...
laboratory evaluation showed the following values: sodium, 139 mEq/L; potassium, 4.0 mEq/L; chloride, 110 mEq/L; bicarbonate, 20 mEq/L; anion gap, 11; serum urea nitrogen, 51 mg/dL; serum creatinine, 3.4 mg/dL (corresponding to eGFR of 18 mL/min/1.73 m²); glucose, 82 mg/dL; calcium, 8.3 mg/dL; phosphate, 6.2 mg/dL; and albumin, 3.8 g/dL.

In the case presented, the patient’s clinical symptoms and biochemistry results improved with relief of constipation, possibly through the decreased generation and increased elimination of uremic toxins. This highlights the importance of colon health in patients with CKD.

**PATHOGENESIS**

**Gut Microbiome in Health**

The human gut harbors ~10^{14} bacteria with an enormous metabolic potential. Under physiologic conditions, the microbiota provide complementary functions by participating in metabolic activities that are not fully evolved in the human host, such as digestion of complex polysaccharides, endogenous synthesis of certain vitamins and amino acids, metabolism of bile acids, degradation of dietary oxalates, and maturation of the immune system.

On average, an individual’s gut microbiota is composed of 500 to 1,000 bacterial species. Findings from the HMP suggest that each individual has a unique microbiome, each niche features one or a few signature taxa, and the gut microbiome is characterized by the greatest diversity with little variation over time. The predominant bacterial groups in the human gastrointestinal tract are Bacteroidetes, Firmicutes, and Actinobacteria. The phylogenetic composition of gut microbiota tends to be similar between individuals living in the same region, belonging to the same family, and having a similar diet. Muegge et al studied the gut microbiome profile in 33 mammalian species, including 18 humans, and reported that the difference in microbiome profiles stems from differing metabolic functions required to utilize the diet. Thus, the gut microbiome appears to change adaptively to the needs of the host organism.

**Gut Microbiome in Kidney Disease**

The term “dysbiosis” was first coined in the early 20th century by the Russian Nobel Laureate Elie Metchnikoff. Dysbiosis is defined as an imbalanced intestinal microbial community with quantitative and qualitative alterations in the composition and metabolic activities of the gut microbiota. Preliminary evidence indicates that the microbiome profile might be altered in patients with chronic kidney failure and earlier stages of CKD (Table 1). Vaziri et al found that 190 microbial operational taxonomic units differed significantly in abundance between patients with end-stage renal disease and apparently healthy controls. Hida et al reported that the number of aerobic bacteria, including Enterobacteria and Enterococci species, is higher in patients treated with maintenance hemodialysis than in controls. Among anaerobic bacteria, Hida et al observed that hemodialysis patients have significantly lower numbers of Bifidobacterium species and higher organism counts for Clostridium perfringens.

The main contributing factors to gut microbiome dysbiosis in patients with kidney disease include slow intestinal transit time, impaired protein assimilation, decreased consumption of dietary fiber, iron therapy, and frequent use of antibiotics. Antibiotic treatment decreases the diversity and alters the relative abundances of members of the bacterial community, with some patients exhibiting incomplete recovery post-treatment.

**Gut-Derived Uremic Toxins and Microbial Metabolites**

In 1965, Einheber and Carter showed that germ-free anephric mice survived longer than anephric mice with an intact gut microbiome. Aronov et al showed that a number of uremic retention solutes are present only in hemodialysis patients with an intact colon. Recently, using untargeted metabolomic mass spectrometry, Wikoff et al reported that the presence of several protein-bound uremic toxins, such as indoxyl sulfate (IS), hippuric acid, and phenylacetic acid, are dependent on the presence of gut microflora.

Impaired protein assimilation in uremia leads to large influx of undigested proteins into the distal intestine, which favors the proliferation of proteolytic bacteria (Fig 1). Increased protein fermentation results in the generation of potentially toxic metabolites, such as ammonia, phenols, amines, indoles, and thiols. Clinical manifestations of these uremic toxins are nonspecific and may include neurologic disorders, protein-energy wasting, cardiovascular disease (CVD), and progression of CKD. The potential pathways linking the accumulation of some of the major toxic metabolites to pathophysiologic consequences in patients with CKD are shown schematically in Fig 2. Increased levels of these toxins in patients with CKD may be related to increased generation from the dysbiotic microbiome or decreased elimination from reduced kidney function. In this review, we focus on the role of the gut microbiome in the generation of uremic toxins (Table 2).

**Ammonia and Urea**

Interdependency between humans and microbes in the metabolic process is exemplified by the urea nitrogen salvage pathway. The end product of mammalian protein catabolism is ammonia, which is toxic to cells in higher concentrations and thus is converted to urea through the ornithine-urea cycle. Mammals cannot break down urea, but gut bacteria expressing...
ESRD patients (n = 5)

Hemodialysis patients (n = 8)

ESRD patients (n = 24) vs healthy persons (n = 12)

ESRD patients (n = 24) vs healthy persons (n = 12)

ESRD patients (n = 10) vs healthy persons (n = 8)

ESRD patients (n = 52)

5/6 nephrectomy (n = 6) or sham-operated rats (n = 5)

Uremic (n = 20) and control (n = 20) Wistar rats

**Human Studies**

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**Experimental Animal Studies**

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**Abbreviations:** CKD, chronic kidney disease; CRP, C-reactive protein; ESRD, end-stage renal disease; GI, gastrointestinal; IL, interleukin; rDNA, ribosomal DNA.

urease cleave urea into ammonia and carbon dioxide. Some of the ammonia can be used for microbial synthesis of amino acids or can enter the host circulation and serve as a substrate for synthetic processes. Ammonia in the large intestine could also be generated by microbial fermentation of glutamine, serine, threonine, and glycine. In a series of studies using CKD rats and human colonocytes, Vaziri et al demonstrated that exposure to ammonia and ammonium hydroxide damages the intestinal epithelial tight junction and impairs its barrier function.

**Creatinine, Guanidine, and Uric Acid**

Jones and Burnett, estimated that about 16% to 60% of orally administered ⁴¹⁴C-labeled creatinine is either metabolized or excreted by routes other than urine in patients with kidney failure. Creatinine concentration in ileal effluent is similar to plasma, but it is undetectable in stool, suggesting that it is possibly degraded by colonic bacteria. Furthermore, 1-methyl guanidine is produced by the metabolism of creatinine by Pseudomonas stutzeri. Guanidine compounds accumulate in CKD, and some of them are considered uremic toxins. Administration of methyl guanidine to rats with kidney failure results in a dose-dependent increase in mortality.

Urate (uric acid) is the end product of purine metabolism. Through excretion, the kidneys play an important role in maintaining serum urate levels, and sustained hyperuricemia in patients with CKD is associated with gout, hypertension, and CVD.

Although the kidney is the primary route for urate excretion, there is a minimal increase in its serum concentration in advanced CKD due to CKD-induced adaptive secretion of uric acid by the colon. Consequently, bacterial families possessing urease, uricase, and enzymes capable of forming indole and p-cresol are expanded in patients with end-stage renal disease.

**Indoles**

Indoles are an aromatic group of compounds containing a pyrrole ring. Metabolism of tryptophan by bacterial tryptophanase generates more than 600...
Indoles in the gut, which are absorbed and sulfate-conjugated in the liver.\textsuperscript{50}

\textit{Indoxyl sulfate.} The IS concentration in the serum is negatively correlated with level of kidney function\textsuperscript{51} and can be used as a predictor of CKD progression.\textsuperscript{52} IS is normally cleared by the proximal tubules of the kidneys, but accumulates in patients with CKD. Cellular transport of IS is mediated by organic anion transporters 1 and 3,\textsuperscript{53} expression of which has been shown to be reduced in experimental models of kidney failure.\textsuperscript{54} Accumulation of IS in renal proximal tubular cells induces nephrotoxicity by activating nuclear factor-\textit{kB} (NF-\textit{kB}) and plasminogen activator inhibitor type 1 expression.\textsuperscript{54,55} Following its administration to uremic rats, IS increases the expression of genes such as tissue inhibitor of metalloproteinases and transforming growth factor \textit{b}1 (TGF\textit{b}1), which are known mediators of tubulointerstitial fibrosis.\textsuperscript{56}

An elevated IS level is associated with aortic calcification, vascular stiffness, and increased risk for overall and cardiovascular mortality in patients with CKD.\textsuperscript{57-59} Experimental studies show that IS enhances oxidative stress in endothelial cells,\textsuperscript{60} increases endothelial microparticle shedding,\textsuperscript{61} impairs the endothelial cell repair mechanism,\textsuperscript{62} and induces vascular smooth muscle cell proliferation.\textsuperscript{39} Interestingly, this indole may decrease erythropoietin production by interfering with oxygen sensing in erythropoietin-producing cells.\textsuperscript{63} Furthermore, IS is taken up by osteoblasts, where it augments oxidative stress and downregulates parathyroid hormone receptor expression, leading to low-turnover bone disease.\textsuperscript{64} IS also has an inhibitory effect on osteoclast function.\textsuperscript{65} In non-dialysis-dependent patients with CKD, IS has been found to be positively associated with bone formation rate.\textsuperscript{56} IS is a ligand of the aryl-hydrocarbon receptor (AhR), a transcriptional regulator that has been shown to cause podocyte injury.\textsuperscript{67} Similar to \textit{p}-cresyl sulfate (PCS), IS is also highly protein bound, preventing it from being effectively removed by hemodialysis.\textsuperscript{68,69}

\textit{Indole acetic acid.} A protein-bound uremic solute, indole acetic acid (IAA) is generated from tryptophan by both intestinal bacteria and normal cells. Plasma IAA concentrations are elevated in CKD and the compound is only partly removed during hemodialysis.\textsuperscript{70} IAA has been shown to induce glomerular sclerosis and interstitial fibrosis in subtotally nephrectomized rats, thus contributing to the progression of CKD.\textsuperscript{71} IAA is also a significant predictor of mortality and cardiovascular events in patients with CKD.\textsuperscript{72} IAA activates the nongenomic AhR pathway, resulting in induction of the proinflammatory enzyme cyclooxygenase 2 and oxidative stress.\textsuperscript{72}

\textbf{Phenols}

Phenols are aromatic compounds with 1 or more hydroxyl groups attached to a benzene ring. Partial breakdown of tyrosine and phenylalanine by several
intestinal bacteria genera, including *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Enterobacter*, and *Clostridium*, generates phenols and p-cresol. Most of the phenols produced in the colon are quickly absorbed and modified by sulfate, acetate, and more rarely, glucuronide conjugation, especially in the liver or colonic mucosa, making them less toxic, and it facilitates their excretion by organic ion transport systems.

*p-Cresyl sulfate*. PCS is a 188-Da uremic retention solute and a uremic toxin. Urinary excretion depends on tubular secretion through specific transporters, leading to progressive accumulation in patients with CKD. In experimental studies, IS and PCS activate the intrarenal renin-angiotensin system, TGF/Smad pathway, and possibly epithelial mesenchymal transformation, leading to fibrosis of the kidney. Watanabe et al showed that PCS administration causes significant renal tubular damage in 5/6-nephrectomized rats by increasing oxidative stress and inflammatory cytokines. An elevated plasma PCS level is associated with all-cause mortality and CVD in patients with chronic kidney failure and earlier stages of CKD. Koppe et al demonstrated the contribution of PCS to CKD-associated insulin resistance and cachexia by its action on adipose tissue and increase of lipolysis. Mice treated with PCS have been shown to display altered insulin signaling in skeletal muscle through activation of ERK1/2.

**Phenylacetylglutamine.** Phenylacetylglutamine is a major microbe-derived nitrogenous metabolite that accumulates in uremia.
<table>
<thead>
<tr>
<th>Solute (MW)</th>
<th>Group</th>
<th>Source</th>
<th>Related Bacteria</th>
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<tr>
<td>Ammonia (17 Da)</td>
<td>Free, Water-Soluble, Low-MW Molecules (&lt; 0.5 kDa)</td>
<td>Bacterial hydrolysis of urea by urease; bacterial fermentation of glutamine, serine, threonine, and glycine</td>
<td>Urease is produced by diverse bacterial species: Clostridium spp, Enterococcus, Shigella, and Escherichia coli</td>
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<td>TMAO (75 Da)</td>
<td>Amine</td>
<td>Endogenous; bacterial metabolism of dietary lipid phosphatidylcholine</td>
<td>Faecalibacterium prausnitzii, Bifidobacterium</td>
<td>† progression of kidney disease and mortality in CKD; † tubulointerstitial fibrosis and collagen deposition; † phosphorylation of Smad3, which regulates profibrotic TGFβ/Smad3 signaling</td>
<td>97-99</td>
</tr>
<tr>
<td>Homocysteine (135 Da)</td>
<td>Amino acid</td>
<td>Endogenous; intestinal bacteria lower homocysteine by production of folic acid</td>
<td>Blifidobacterium spp</td>
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<td>D-Lactic acid (90 Da)</td>
<td>D-Amino acid</td>
<td>Ingestion; endogenous; bacterial production</td>
<td>Enterococcus and Streptococcus spp Oxalobacter formigenes, Blifidobacterium lactis, Enterococcus faecalis, and Eubacterium lentum</td>
<td>D-Lactic acidosis; neurotoxic effects; encephalopathic symptoms</td>
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<td>Oxalate (90 Da)</td>
<td>Phenol</td>
<td>Ingestion; endogenous; certain intestinal bacteria have oxalate-degrading potency</td>
<td>Closstridium difficile, F prausnitzii, Blifidobacterium, Subdoligranulom Lactobacillus</td>
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<td>p-Cresyl sulfate (188 Da)</td>
<td>Phenol</td>
<td>Bacterial metabolism of tyrosine and phenylalanine</td>
<td>Closstridium sporogenes, Escherichia coli</td>
<td>† progression of CKD, CVD, and mortality in hemodialysis patients; † cytokine-stimulated expression of endothelial adhesion molecules; † endothelial permeability</td>
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<td>Indoxyl sulfate (213 Da)</td>
<td>Indole</td>
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<td>Closstridium sporogenes, Clostridium bartletti, E coli</td>
<td>† vascular stiffness, aortic calcification, and cardiovascular mortality; † oxidative stress in endothelial cells, † vascular smooth muscle cell proliferation; † expression of genes related to tubulointerstitial fibrosis; † nephrotoxicity</td>
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<td>Indole-3-acetic acid (175 Da)</td>
<td>Indole</td>
<td>Endogenous; bacterial metabolism of tryptophan</td>
<td>Closstridium sporogenes, Clostridium bartletti, E coli</td>
<td>Induce glomerular sclerosis and interstitial fibrosis in subtotally nephrectomized rats, thus contributing to progression of CKD; serum indole-3-acetic acid is a significant predictor of mortality and cardiovascular events in patients with CKD; induces proinflammatory enzyme COX-2 and oxidative stress</td>
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(Continued)
derived from β-phenylethylamine formed in the large intestine by decarboxylation of phenylalanine released by bacterial proteolysis of unabsorbed protein. Phenylacetylglutamine is also produced in the liver by the metabolism of phenylacetic acid, derived from phenylalanine. The precursor phenylacetic acid itself is toxic and induces nausea, vomiting, diarrhea, and convulsion. The latter is associated with impaired immunoregulation, increased oxidative stress, and osteoblast dysfunction. Autopsy studies in dogs have shown that exposure to phenylacetic acid causes renal tubular damage and thus may contribute to the progression of kidney disease.

### Hippurate

Gut microbial metabolism generates benzoate from dietary aromatic compounds, and the subsequent hepatorenal conjugation of benzoate with glycine forms hippurate. Hippurate is generally believed to be nontoxic, except for contributing to anion gap acidosis. There is some evidence that hippurate may cause glucose intolerance and interfere with erythropoiesis and platelet cyclooxygenase activity.

### Amines

**Polyamines.** Generated by the gut microbiota from precursor amino acids, polyamines include putrescine, agmatine, cadaverine, tyramine, and histamine. Igarashi et al observed a decrease in spermine and an increase in putrescine, as well as acrolein (a major toxic compound deriving from spermine), in plasma of patients with CKD. Cellular downregulation by polyamines is proposed to play a role in the lack of tissue response to hormones in uremia. Polyamine also inhibits the activity of erythropoietin and hence may play a role in the anemia of CKD.

**D-Amino acids.** Among other potential uremic toxins are some of the D-amino acids. It has been shown that plasma levels of certain D-amino acids increase as GFR declines. D-Lactic acid originates from endogenous production by the methylglyoxalase pathway or as a byproduct of bacterial metabolism in the intestine. Oh et al first described D-lactic acidosis, which has been associated with neurotoxicity.

### Recent Advances

**Trimethylamine-N-Oxide**

In a landmark study, Wang et al performed metabolomic studies and screened more than 2,000
compounds in 75 patients with CVD and identified trimethylamine-N-oxide (TMAO), choline, and betaine as being associated with heart disease. In a study involving 4,007 patients undergoing elective coronary angiography, an elevated TMAO level was found to predict increased risk for major adverse cardiovascular events after adjustment for traditional risk factors. Choline is catabolized by the intestinal microbiota to form trimethylamine gas, which is subsequently metabolized by the liver into TMAO. Dietary carnitine, which is found in red meat, is another substrate for gut flora to produce TMAO.

Microbial taxa belonging to the Clostridiaceae and Peptostreptococcaceae families are positively associated with blood TMAO levels in humans. The way in which TMAO promotes atherosclerosis remains speculative, but it has been shown to cause alteration of cholesterol and sterol metabolism, promote foam cell formation by increasing the expression of scavenger receptors on macrophages, and lead to alterations in bile acid metabolism and sterol transporters in the liver and intestine. A high TMAO level is a predictor of increased long-term mortality in patients with heart failure, which is independent of traditional risk factors.

Plasma TMAO levels are elevated in patients with CKD and are associated with increased risk for death. In animal models, feeding with TMAO and choline leads to tubulointerstitial fibrosis and collagen deposition, which is accompanied by a significant increase in the phosphorylation of Smad3.

Hydrogen Sulfide

Sulfate-reducing bacteria in the human colon can use hydrogen gas or organic compounds as electron donors for reduction of sulfate or other oxidized sulfur compounds to generate hydrogen sulfide (H2S). This toxic gas belongs to the family of gasotransmitters and inhibits mitochondrial respiration through the blockade of cytochrome c oxidase, with genotoxic, cytotoxic, and inflammatory effects. Despite these reports of toxicity, findings from animal models of ischemia-reperfusion injury and heart failure suggest that H2S could be cardioprotective. In animal models of CKD plasma, H2S production by the kidney and liver is reduced due to downregulation of H2S-producing enzymes. Preliminary evidence indicates that plasma H2S level is reduced in hemodialysis patients compared with controls. In in vitro studies, sodium H2S, an H2S donor, decreases inflammation and inhibits renal fibrosis, possibly through inhibiting the TGFβ1/Smad and mitogen-activated protein kinase signaling pathways. The potential beneficial effect of H2S in CKD and whether it retains its toxicity in higher concentrations need further study.

Endotoxin

Endotoxin is a phospholipid that forms the outer membrane of most Gram-negative bacteria. Circulating endotoxin binds lipopolysaccharide binding protein (LBP), and together these proteins interact with MD-2, which forms a complex with Toll-like receptor 4, anchored by CD14. This binding stimulates, by activation of NF-κB, the translation and production of inflammatory cytokines. Endotoxin translocation from the gut has been suggested as one of the causes of inflammation in CKD. Furthermore, endotoxin is known to play an important role in the initiation and progression of atherosclerosis by mediating endothelial cell injury, boosting monocyte recruitment, transforming macrophages to foam cells, and activating coagulant activity. We have demonstrated that soluble CD14 is associated with progression of CKD, CVD, and mortality in patients with kidney disease. Furthermore, endotoxin has been identified as an inflammatory trigger of insulin resistance, obesity, and diabetes in mice.

Gut Microbe–Derived Mediators of Immune Regulation

It now is evident that gut microbes play a key role in shaping the human immune system. Polysaccharide A produced by Bacteroides fragilis induces the accumulation of Foxp3-positive regulatory T cells and production of interleukin 10. Another molecule that is derived from gut microbiota and can modulate peripheral immune function is peptidoglycan, an essential component of the cell wall of virtually all bacteria. Upon entry to blood, peptidoglycan systemically primes the innate immune system. Peptidoglycan has been shown to signal by the pattern-recognition receptor Nod1. The role of these and other microbe-derived molecules in mediating dysregulated immune response in patients with CKD warrants further exploration.

Short-Chain Fatty Acids

An altered dysbiotic gut microbiome will not only produce an array of harmful metabolites and uremic toxins, but can also potentially cease to produce the otherwise beneficial metabolites such as short-chain fatty acids (SCFAs). These 1- to 6-carbon aliphatic organic acids are the products of anaerobic bacterial fermentation of dietary polysaccharides and include acetate, propionate, and butyrate. SCFAs enter the systemic circulation through colonocytes by passive diffusion and active transport mechanisms. They affect a range of host functions, including energy metabolism, immune regulation and gut motility, and blood pressure regulation through activation of G protein–coupled receptors such as GPR41 and GPR43.
Butyrate is the primary source of energy for colonicocytes and is thus associated with maintenance of the epithelium. More recently, researchers have expanded the role of SCFAs to explain the gut-kidney connection in ischemia-reperfusion injury, showing that treatment with SCFAs reduces kidney injury of this type in a germ-free mouse model system. The key mechanism protecting against acute kidney injury was suggested to be a reduction in inflammation mediated by an epigenetic mechanism. If SCFAs are important in acute kidney injury, it might be of interest to also define their role in the progression of CKD.

Interestingly, SCFAs may also influence blood pressure through activation of GPR41 and GPR43, which are expressed in adipocytes, neutrophils, and sympathetic ganglia. Olfactory receptor 78 (Olfr78) expressed in the kidney responds to SCFAs where it mediates renin secretion and increases blood pressure; this increase is counteracted by GPR43, which induces vasodilatation.

Advances in Technology and Discovery of Uremic Toxins

In 2003, the European Uremic Toxin Work Group catalogued 90 different uremic retention solutes, and this list has since grown to more than 150. The discovery of the newer solutes is primarily driven by the advent and implementation of “omic” technologies such as metagenomics, transcriptomics, proteomics, and metabolomics.

It is apparent that uncultured microorganisms represent ~99% of the gut microbiota. Fortunately, because 16S ribosomal RNA sequences are highly conserved within organisms of the same genus and species, they can be used to determine phylogeny. Metagenomics is culture-independent analysis of microbial genomes, which allows understanding the dynamics and diversity of the microbial community and its interaction with hosts.

In the search for uremic toxins, the use of proteomics and metabolomics could be complementary. Proteomics focuses on the study of peptides and proteins, whereas metabolomics is useful in the identification of small metabolites (<1,000 Da) such as amino acids, alcohols, vitamins, polyols, and organic acids, as well as nucleotides. Because metabolites are downstream of both transcription and translation, they may be more reflective of disturbed metabolism than proteins, messenger RNA, and genes, but each technique has a unique role in the discovery of uremic retention solutes.

Metabolite profiling of plasma samples from 1,434 Framingham study participants demonstrated that 9 metabolites predict the development of CKD. Interestingly, choline was 1 of the 3 markers that remained significant after adjustment for eGFR, age, sex, diabetes mellitus, hypertension, and proteinuria at baseline. A metabolomic study in patients with stages 3 to 4 CKD revealed that levels of 14 metabolites were elevated in uremic plasma. In addition to confirming the retention of several previously identified uremic toxins, including PCS, this study detected 2 novel uremic retention solutes, dimethyl sulfone and 2-hydroxyisobutyric acid. It is essential to emphasize the importance of investigating the underlying biochemical mechanisms of any newly discovered uremic solute in order to determine its pathophysiologic importance.

The Microbiome as a Therapeutic Target

Unlike the human genome, the gut microbiome has a dynamic composition that is susceptible to manipulation and selective “farming” of desired microbial populations for the benefit of the human host. As we unravel the details of interactions between the host and the microbiota, as well as those within the microbiota itself, new classes of therapeutics will emerge to harness the vast therapeutic potential of this extraordinary natural resource. Among the seemingly limitless potential applications of human gut microbiome, its use as disease biomarker, alteration of the microbiome composition to treat disease, and genetic engineering of the microbes to gain new functions or deliver small therapeutic molecules have been proposed.

Gut Microbiome–Based Biomarkers

Gut microbiome composition has been considered as a potential tool for diagnosing, monitoring, and prognosticating diseases. The microbiome profile is not only altered in CKD, it also varies by underlying cause. Whether the dysbiotic microbiome is a cause and/or consequence of CKD is not clear. The potential utility of the gut microbiome as a biomarker for screening individuals with susceptibility to develop CKD remains to be investigated.

Manipulation of the Microbiome Composition

Improved understanding of the gut microbiome’s physiologic functions and the pathologic effects of dysbiosis has triggered interest in various ways of re-establishing symbiosis. The generation of uremic toxins could be reduced by selectively increasing saccharolytic bacteria and reducing proteolytic bacteria in the colon. A number of therapeutic interventions have been explored to modulate gut microbiota, or adsorption of uremic toxin end products of microbial fermentation (Table 3).

Probiotics are defined as “live microorganisms” that when administered in adequate quantities bestow a health benefit on the host. However, administration of an enteric capsule preparation of *Bifidobacterium longum* to patients with CKD was reported to have only minimal effects on CKD progression. In a randomized, double-blind, placebo-controlled, crossover study...
involving 22 hemodialysis patients, Renadyl (a specific probiotic formulation; Kibow Biotech) was not observed to have an effect on microbe-derived uremic toxins.  

Despite these negative findings and the absence of large-scale studies, a recent randomized double-blind placebo-controlled trial involving 39 peritoneal dialysis patients reported significant reductions in serum levels of endotoxin and proinflammatory cytokines, increases in serum levels of an anti-inflammatory cytokine (interleukin 10), and preservation of residual kidney function following a 6-month probiotic therapy. In another double-blind randomized placebo-controlled trial with 30 patients with CKD (stages 3–4), the symbiotic Probinul neutro (CaDiGroup Srl) was shown to significantly lower total plasma p-cresol concentrations after 2 to 4 weeks of treatment. One of the main limitations to probiotic therapy is that no study has yet demonstrated sustained survival of probiotics in the colon of dysbiotic patients with CKD.

Table 3. Treatment Options for Uremic Toxins and Inflammation

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Patient Type</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic Oligofructose-enriched inulin</td>
<td>Healthy participants (n = 50)</td>
<td>↓ Urinary excretion of p-cresol</td>
<td>72</td>
</tr>
<tr>
<td>Inulin/oligofructose</td>
<td>Obese women (n = 30)</td>
<td>↓ Endotoxemia</td>
<td>64</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Elderly participants (n = 74)</td>
<td>↓ TNF-α mRNA and IL-6 mRNA; ↓ serum soluble CD14</td>
<td>73</td>
</tr>
<tr>
<td>Probiotic</td>
<td>Peritoneal dialysis patients (n = 39; 21 in probiotics group and 18 in placebo group)</td>
<td>↓ TNF-α, IL-5, IL-6, and endotoxin; ↑ IL-10; preserve residual kidney function</td>
<td>138</td>
</tr>
<tr>
<td>Synbiotic Oligofructose-enriched inulin</td>
<td>Hemodialysis patients (n = 22)</td>
<td>↓ Serum p-cresyl sulfate and generation rate</td>
<td>58</td>
</tr>
<tr>
<td>Probiotic along with inulin, oat bran, pectin, and resistant starch</td>
<td>Trauma patients (n = 65)</td>
<td>↓ Rate of systemic inflammatory response, syndrome, infections, severe sepsis, and mortality</td>
<td>74</td>
</tr>
<tr>
<td>Galacto-oligosaccharides, Lactobacillus casei, and Bifidobacterium breve</td>
<td>Hemodialysis patients (n = 7)</td>
<td>↓ Serum p-cresol</td>
<td>143</td>
</tr>
<tr>
<td>Probinul neutro</td>
<td>CKD patients stages 3-4 (n = 30)</td>
<td>↓ Total plasma p-cresol</td>
<td>139</td>
</tr>
<tr>
<td>Oral adsorbent AST-120</td>
<td>Adult patients with moderate to severe CKD (n = 132)</td>
<td>↓ Indoxyl sulfate in a dose-dependent manner; unable to demonstrate beneficial effect of AST-120 on progression of CKD</td>
<td>146,148</td>
</tr>
<tr>
<td>α-Galactosidase Inhibitor Acarbose (an inhibitor of the α-glucosidase enzymes)</td>
<td>Healthy volunteers (n = 9)</td>
<td>↓ Serum concentrations of p-cresol; ↓ urinary excretion of p-cresol; ↑ fecal excretion of nitrogen increased</td>
<td>160</td>
</tr>
<tr>
<td>Genetically Engineered Bacteria Microencapsulated genetically engineered live Escherichia coli DH5 cells</td>
<td>Uremic rats</td>
<td>↓ Plasma urea concentration; ↓ plasma ammonia concentration</td>
<td>145</td>
</tr>
<tr>
<td>Ongoing Studies SYNERGY-HMW inulin, fructo-oligosaccharides and galacto-oligosaccharides plus strains from the Lactobacillus, Bifidobacteria, and Streptococcus genera</td>
<td>Patients with moderate to severe CKD stages 4-5 (n = 37)</td>
<td>Ongoing study aiming to assess effectiveness of synbiotics on the synthesis of uremic toxins indoxyl sulfate and p-cresyl sulfate</td>
<td>149</td>
</tr>
<tr>
<td>Arabinoxylan-oligosaccharides (AXOS)</td>
<td>Patients with CKD stage 3b-4</td>
<td>Ongoing study aiming to assess whether AXOS can decrease intestinal generation and serum concentrations of microbial metabolites in patients with CKD</td>
<td>161</td>
</tr>
</tbody>
</table>

Abbreviations: CKD, chronic kidney disease; HMW, high-molecular-weight; IL, interleukin; mRNA, messenger RNA; TNF-α, tumor necrosis factor α.
Furthermore, care should be taken when choosing probiotics because the contribution of bacteria with the ability to hydrolyze urea may be more harmful.

Prebiotics are nondigestible food ingredients for which the positive effects are due to stimulating the growth or activity of one or a limited number of bacteria in the colon. Meijers et al.\textsuperscript{140} showed that oligofructose inulin significantly reduces PCS generation rates and serum concentrations in hemodialysis patients, but has no effect on IS. In contrast, a randomized controlled trial showed that resistant starch decreased IS levels in patients treated with hemodialysis.\textsuperscript{141} In rats, a high amylose-resistant starch diet was observed to retard CKD progression and attenuate oxidative stress and inflammation.\textsuperscript{142} A small study of hemodialysis patients showed that a synbiotic (combining *Lactobacillus casei* and *Bifidobacterium breve*, and galacto-oligosaccharides) decreased *p*-cresol but not IS levels.\textsuperscript{143}

**Genetic Engineering of the Gut Microbes**

Genetic engineering of bacteria for the delivery of drugs and small therapeutic molecules holds tremendous potential as a future approach to treat a wide range of diseases and promote health. Prakash and Chang\textsuperscript{144} used microencapsulated *Escherichia coli* cells genetically engineered to contain a *Klebsiella aerogenes* gene and showed that these modified bacteria were able to effectively remove urea and ammonia in vitro and lower plasma creatinine levels in rats when given orally on a daily basis.\textsuperscript{145} At present, safety concerns prevent the release of genetically engineered cells into the body, and the field anxiously welcomes further pioneering research to improve our knowledge of the gut microbial ecology so that it can make more educated attempts to manipulate the gut microbial community for therapeutic purposes.

Another method for decreasing gut-derived uremic toxins is to use oral sorbents. The oral sorbent AST-120 was found to decrease plasma IS levels in a dose-dependent manner.\textsuperscript{146} The primary effect of AST-120 appears to be mediated through its adsorption of urea-derived ammonia and interruption of enterohepatic urea recycling.\textsuperscript{147} Although small randomized controlled animal studies have suggested a renoprotective effect for AST-120, a subsequent large randomized controlled trial could not confirm it.\textsuperscript{148}

The larger study has some methodological limitations, but it also raises the possibility that targeting specific microbiome-derived uremic toxin may not be sufficient because the microbiomes generate a myriad of yet unidentified toxins.

Thus, the quality of evidence emerging from the small, uncontrolled, mostly single-center studies targeting the microbiome needs careful scrutiny, and these results need replication in well-designed large-scale multicenter trials. Among the currently ongoing studies, a few are appropriate to highlight. In a double-blind, placebo-controlled, randomized, crossover trial, Rossi et al.\textsuperscript{149} is recruiting 37 patients with CKD for a 6-week synbiotic therapy (or placebo) to determine the efficacy of synbiotic therapy for lowering serum IS and PCS levels. The National Institute of Diabetes and Digestive and Kidney Diseases is proposing to conduct 2 highly intensive phase 2, randomized, double-blind, placebo-controlled, multicenter studies examining the effect of prebiotic therapy on the microbiome and the associated metabolomics profile in patients with CKD not yet on dialysis therapy and maintenance hemodialysis patients. These studies are expected to be launched shortly.

**SUMMARY**

It is becoming evident that interaction of the human host with our resident microbes at the gene, protein, and metabolite levels could have a significant impact on health and disease. The features and composition of the gut flora reflect selective forces acting on both the microbial community and the host. Whether an adaptive change in the microbiome in response to the uremic...
state becomes maladaptive, leading to increased generation of uremic toxins and associated complications, needs to be rigorously examined using modern techniques in clinical and experimental settings. It is also possible that the presence of a specific microbiome or absence of a protective microbiome increases the susceptibility to kidney disease when exposed to injury (Fig 4). Supported by preliminary evidence and enticed by the novelty of the concept, a number of interventions have been proposed to reduce uremic toxicity targeting the gut microbiome and have been tested with mixed results. The findings from these studies should be viewed cautiously and further examined in well-designed large-scale studies prior to implementation. Needless to say, if proved to be effective, gut microbiome–targeted interventions will have a significant impact on the management of patients with chronic kidney failure and earlier stages of CKD.

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