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Probiotics and chronic kidney disease

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Probiotics are the focus of a thorough investigation as a natural biotreatment due to their various health-promoting effects and inherent ability to fight specific diseases including chronic kidney disease (CKD). Indeed, intestinal microbiota has recently emerged as an important player in the progression and complications of CKD. Because many of the multifactorial physiological functions of probiotics are highly strain specific, preselection of appropriate probiotic strains based on their expression of functional biomarkers is critical. The interest in developing new research initiatives on probiotics in CKD have increased over the last decade with the goal of fully exploring their therapeutic potentials. The efficacy of probiotics to decrease uremic toxin production and to improve renal function has been investigated in *in vitro* models and in various animal and human CKD studies. However to date, the quality of intervention trials investigating this novel CKD therapy is still lacking. This review outlines potential mechanisms of action and efficacy of probiotics as a new CKD management tool, with a particular emphasis on uremic toxin production and inflammation.

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Chronic kidney disease (CKD) is emerging as a major risk factor of cardiovascular disease (CVD). Uremic illness is considered to be due to the accumulation of organic waste products, so-called uremic retention solutes (URSs) that are normally cleared by the kidneys. URS such as phenols and indoles are generated along the gastrointestinal tract (GIT), where the gut microbiota has a significant role in their production[1] and have been shown to have deleterious effects on the cardiovascular system. A number of treatments targeting URS have been proposed, such as reducing substrates (dietary protein restriction), decreasing absorption (oral adsorbents such as AST-120), increasing clearance by renal replacement therapies (long and/or more efficient dialysis, absorbent membranes, kidney transplantation), and modulating cellular pathways (organic anion transporters and antioxidants).[2] Unfortunately, most of these treatments display inherent disadvantages (side effects, high cost, unavailability in patients with moderate CKD) and remain limited to experimental studies.

The gut microbiota is essential for regulating the normal function of the intestinal barrier: it promotes immunological tolerance to antigens from nutrients or organisms, controls nutrient uptake and metabolism, and prevents propagation of pathogenic organisms.[3] Hence, the concept has emerged that dysregulation of intestinal microbiota may have a significant role in cancer and metabolic and inflammatory digestive disease. Recently, it has been demonstrated that CKD is associated with dysbiotic gut microbiotia.[4]

During CKD, the potential utilization of therapies modulating the gut microbiota such as probiotics has emerged as an attractive strategy to reduce URS and improve CVD. Probiotics, a word derived from Greek meaning 'for life', is defined by the World Health Organization[5] as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'. Probiotics are being increasingly used for various pathologic conditions.[6] However, not all probiotics strains are beneficial in all circumstances and the careful selection of specific organisms based on desired clinical outcome is crucial. Over the past 15 years, considerable experimental and clinical data reinforced the hypothesis that probiotics have a therapeutic role in maintaining a metabolically balanced GIT, reducing the progression of CKD and the generation of URS. For the purpose of this review, we will define the mechanisms of the action of probiotics and we will focus on recent

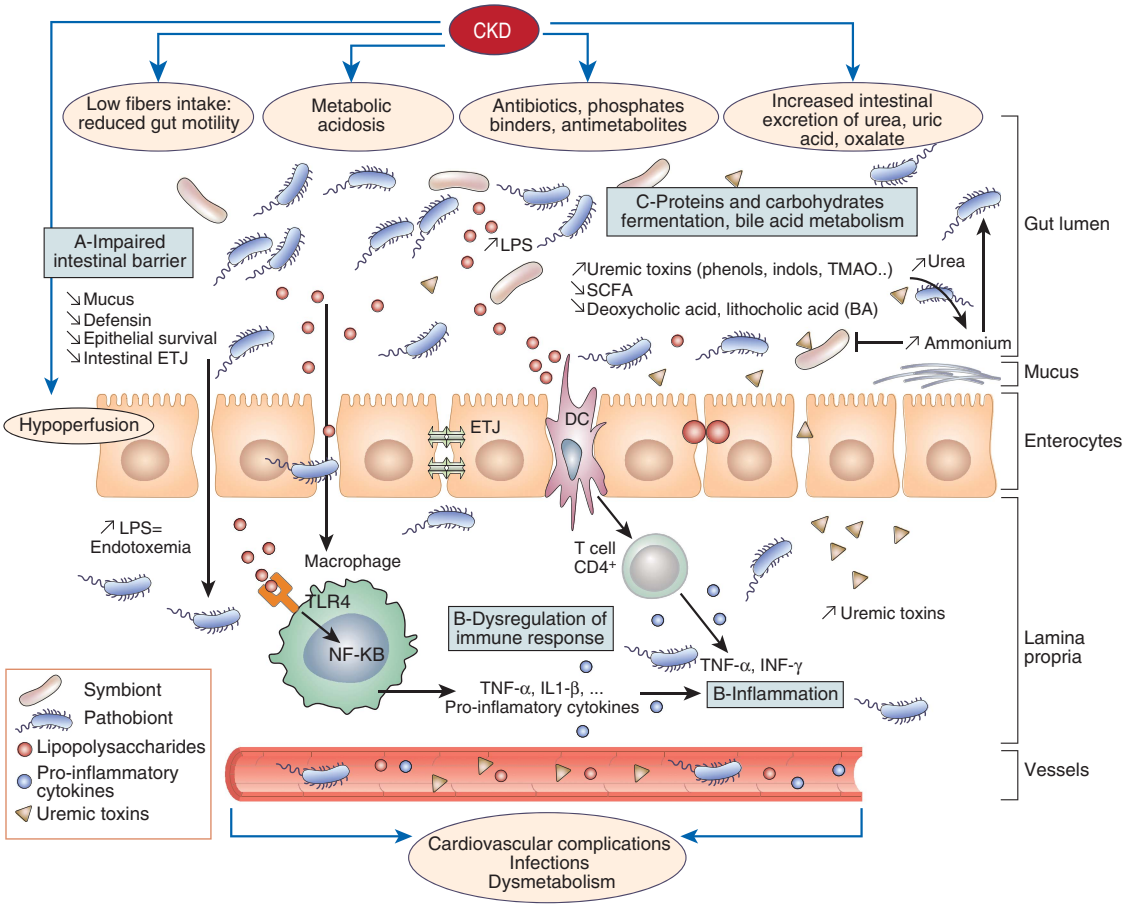

Figure 1 | Dysbiosis and chronic kidney disease (CKD). CKD impairs the balance between symbionts and pathobionts in a way that favors pathobionts overgrowth. Consequences are as follows: (A) impairment of intestinal barrier by disrupting the colonic epithelial tight junction (ETJ) and decreasing epithelial survival. Loss of integrity increase in intestinal permeability allows translocation of bacteria and lipopolysaccharide (LPS). (B) Dysregulation of immune response and inflammation. LPS could activate innate immune cells through a Toll-like receptor 4 (TLR4)-dependent and nuclear factor-kappa B (NF-κB) pathways. Pathobionts stimulate dendritic cells (DCs) that activate a Th17/Th1 T-cell response and enhance production of inflammatory cytokines. (C) Modification of carbohydrates, protein, and bile acid (BA) fermentation. Proteins are fermented by intestinal pathobionts, which are then converted preferentially into indoxyl-sulfate (IS), p-cresyl sulfate (PCS), and trimethylamine n-oxide (TMAO). The reduction in symbionts, specifically *Bifidobacterium,* induces a decrease in short-chain fatty acids (SCFAs). Dysbiosis modifies BA levels and composition. INF-γ, interferon γ; IL-1, interleukin-1; TNF-α, tumor necrosis factor-α.

developments in probiotics in the field of CKD from both *in vitro* and *in vivo* studies.

DYSBIOSIS AND CKD

Recent data highlight that uremia is associated with abnormalities in the gastrointestinal mucosa[7] and a disequilibrium in the intestinal ecosystem.[4] Specifically, these studies demonstrate the presence of aerobic bacteria, such as *Firmicutes*, *Actinobacteria,* and *Proteobacteria,* and fewer anaerobic bacteria, such as *Sutterellaceae*, *Bacteroidaceae*, and *Lactobacillaceae.*[4] The intestinal dysbiosis may be due to iatrogenic causes or uremia *per se* as shown in Figure 1. If the consequences of intestinal microbiota dysregulation in the progression and complications of CKD are currently largely unknown, recent studies give new insights.

First, besides the passive accumulation of URS due to a reduction in kidney clearance, the modification of the intestinal microbiota in CKD strongly increases transformation of amino acids into URS, e.g., indoxyl-sulfate (IS), p-cresyl sulfate (PCS), and trimethylamine n-oxide (TMAO)[1] among others. Increased intestinal concentration of uremic toxins may lead to microbial dysbiosis and pathobionts overgrowth. For example, a modification of the GIT biochemical milieu in the presence of locally accumulated uric acid and urea could perturb symbionts overgrowth.[8] Second, the dysbiosis could participate in immune dysregulation and inflammation in CKD.[9] Pathobionts trigger the intestinal immune system toward a proinflammatory response by preferentially activating Th17-Th7 cells and increasing the production of lipopolysaccharides (LPSs), a major component of the outer membrane of Gram-negative bacteria. Third, dysbiosis also contributes to an increase in intestinal permeability by disrupting the colonic epithelial tight junction,[7] which may subsequently lead to translocation of LPS and bacteria into the host's internal environment. Finally, metagenomic analyses of the microbiota performed in

obese populations revealed an increase in *Firmicutes* and reduced *Bacteroidetes* similar to what has been described in CKD patients.[4] It is therefore possible that the modification of intestinal microbiota in CKD might be involved in insulin resistance and dyslipidemia through increased LPS production, modified carbohydrate fermentation or bile acid level and composition.[10,11] Given that gut-derived uremic toxins, inflammation and insulin resistance contribute to progression of CKD as well as CVD, dysbiosis could have an important role in mortality in CKD.[1,11,12]

PROBIOTICS AND HEALTH

Definition

The term probiotic is often misused, which has led to the marketing of products that exploit this term. In 2014, the International Scientific Association for Probiotics and Prebiotics established a consensus statement clarifying the scope of and the appropriate use for the term 'probiotic'.[13] The consensus definition is that probiotics are natural or genetically modified microorganisms expressing specific exogenous enzymes that are able to survive stomach acid and bile, to increase the colon concentration of symbiotons, and confer a health benefit.[5]

Figure 2 summarizes the overall beneficial effects of probiotics and those potentially effective in CKD. Although multiple mechanisms are often represented in a single strain, no individual probiotic would be expected to have all the effects listed in Figure 2. Other effects at the intestinal or the extraintestinal level, including immune and metabolic effects, are more likely to be strain specific.[3]

Probiotics and mucosal effects

Even though the mechanisms regulating epithelial responses to probiotics are complex and poorly understood, the presumed first target of probiotic action is the intestinal epithelial cell through enhancement of epithelial integrity. Some strains may block pathogen entry into the epithelial cell by providing a physical barrier, referred to as colonization resistance, and competition for a limited niche, thereby excluding a site for replication by pathogens. For example, *Lactobacillus helveticus* possesses hydrophobic cell surface properties and therefore is able to nonspecifically bind to intestinal cells.[14] In addition, most probiotics create a mucus barrier by increasing mucin synthesis and secretion from goblet cells.[15]

Probiotics may enhance cell survival and decrease apoptosis during various intestinal assaults.[16] In fact, soluble factors secreted by *Lactobacillus rhamnosus* were found to activate protein kinase B in a phosphatidylinositol-3′-kinase-dependent manner and prevent cytokine-induced apoptosis in human and mouse intestinal cells.[16] *Lactobacillus rhamnosus* is able to produce soluble proteins (p40 and p75), which protect the intestinal barrier from hydrogen peroxide–induced insult.[17] Other probiotics maintain intestinal integrity by increasing the intercellular apical epithelial tight junction via the upregulation of zonula occludens-1 expression or by preventing epithelial tight junction protein redistribution.[18] The protective effects of probiotics on intestinal function have been confirmed in *in vivo* studies using *Citrobacter rodentium* infection in a mouse model of bacterial-induced infectious colitis.[19] This observation should be considered in clinical studies in CKD patients who frequently present with a chronic inflammation of the GIT and where probiotics could enhance the mucosal barrier function.

Probiotics and antimicrobial effects

Several studies have confirmed that probiotics might reduce digestive infection.[3] This is of particular interest as CKD patients are at higher risk of *Clostridium difficile* infection.[20] Indeed, some probiotic strains have been shown to produce elaborated antibacterial compounds referred to as bacteriocins or antimicrobial peptide. Antimicrobial peptides may act as colonizing peptides, facilitating the competition of a probiotic with the resident microbiota, as killing peptides eliminating pathogens, or serve as signaling peptides for other bacteria or the immune system. Along the same line, lactic acid-producing *Lactobacilli* exert antimicrobial effects by reducing the local pH in the gut lumen.[21] *Lactobacillis salivarus* produces an *in vivo* bacteriocin that has been shown to significantly protect mice against infection with the invasive foodborne pathogen *Listeria monocytogenes*.[22] Finally, *Lactobacillus fermentum* stimulates human β–defensin mRNA expression and protein secretion in the intestine.[23]

Other probiotics could influence gene expression of microbial pathogens and thereby reduce their hostility. For instance, *Lactobacillus acidophilus* may interfere with the virulence gene expression of enterohemorrhagic *Escherichia coli* O157:H7.[24] Probiotics could prevent the binding of enteric pathogens to mucosal surfaces by obscuring the receptor-binding sites, thus preventing pathogens from invading the host and allowing for an increased clearance of the pathogen from the GIT.[25]

Probiotics, immunity, and inflammation

By decreasing the presence of pathobionts, probiotics have proven that it is possible to enhance both innate and adaptive arms of the host immune system.[26] For instance, some probiotic strains have the ability to promote the differentiation of B cells and increase the production of secretory IgA. Polymeric IgA sticks to the mucus layer overlying the gut epithelium and binds to pathogenic microorganisms, thereby reducing their ability to gain access to the endothelial cells.

Other probiotic strains stimulate the innate immune system by signaling to dendritic cells, which then travel to mesenteric lymph nodes where they induce regulatory T cells ($FoxP3^+$) and the production of anti-inflammatory cytokines (interleukin-10 and transforming growth factor-β). For example, *Saccharomyces boulardii* was shown to reduce intestinal inflammation through modulation of the T-cell response and reduced trafficking of Th1 cells, which resulted in a reduction of the proinflammatory cytokine interferon-γ.[27] The relative serum cytokine profiles have been reported to predict the ability of the probiotic strains to have an impact on disease outcome.[28]

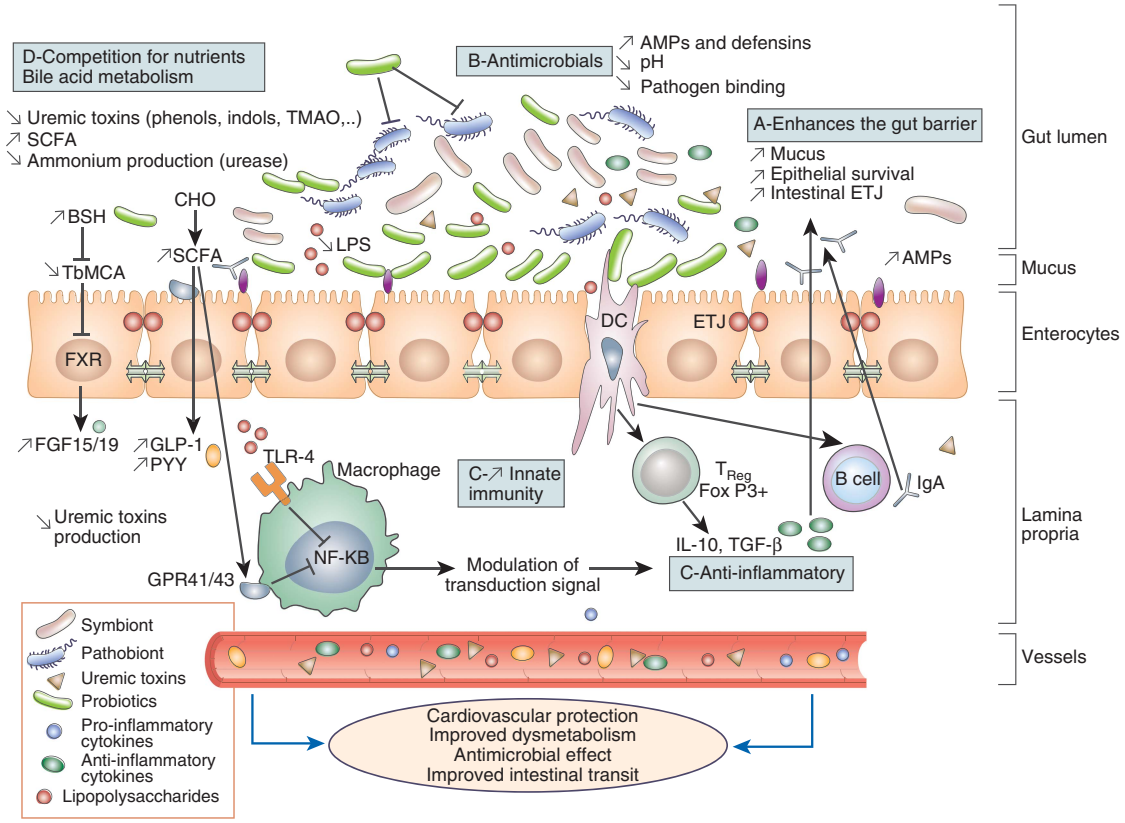

Figure 2 | Potential health benefits of probiotics in chronic kidney disease. Probiotics are live microorganisms able to survive the gastrointestinal tract (GIT) and restore the intestinal flora balance. Their beneficial effects are as follows: (A) Enhanced gut barrier by increasing mucus integrity, epithelial tight junction (ETJ), and epithelial cells survival. (B) Antimicrobial through a reduction in local pH, a production of antimicrobial peptides (AMPs), and defensins by probiotics, which control pathobionts overgrowth. Probiotics could stimulate production of secretory IgA providing additional protection from the luminal microbiota. (C) Anti-inflammatory effect and improved immunity tolerance. Symbionts and probiotics interact with dendritic cells (DCs) and macrophages through pattern-recognition receptors such as Toll-like receptors (TLRs), which signal to the adaptive immune cell as regulatory T cell (T_{Reg}) and B cells. The decrease in lipopolysaccharide (LPS) production reduces the activation of marcrophages and nuclear factor-kappa B (NF-κB) cascade. (D) Competition for nutrients and bile acid metabolism. The reduction in pathobionts limits production of gut-derived uremic toxins. The presence of probiotics increases bile salt hydrolase (BSH) activity, which decreases the abundance of TbMCA and SCFAs production. CHO, carbohydrates; FGF 15/19, fibroblast growth factor 15/19; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; GPR41/43: G-protein-coupled receptors 41/43; IgA: immunoglobulin A; IL-10, interleukin-10; PYY, peptide YY; TbMCA, tauro-beta-muricholic acid; TGF-β, transforming growth factor-β.

Probiotics can also modulate the activation of the proinflammatory nuclear factor-κB to slow down the deleterious LPS flow and decrease interleukin-8 secretion, which is a potent neutrophil chemoattractant to sites of intestinal injury.[26] However, there are also reports that some strains of probiotics are able to activate nuclear factor-κB and increase levels of the proinflammatory cytokine directly or through the increase of ammonia and ammonium hydroxide (NH_3/NH_4OH) production. These discrepancies serve to further emphasize the strain-specific effects of probiotics on the host[26] (Figure 3).

Probiotics and host metabolism

Numerous reports have demonstrated that manipulating the gut microbiota with probiotics, particularly *Lactobacillus* strains, have beneficial effects such as improving glucose homeostasis and reducing inflammation and hepatic steatosis.[29] Probiotics may modify the bile acid profile in the gut. Sayin *et al.* clearly demonstrated that colonization by a transformed bacterium that increases bile salt hydrolase activity influences host metabolic processes by decreasing the abundance of tauro-beta-muricholic acid, a potent antagonist of farnesoid X receptor, resulting in a fibroblast growth factor 15/19–mediated regulation of hepatic cholesterol synthesis and improved metabolic perturbations.[30] Because numerous well-known probiotics exhibit bile salt hydrolase activity, this may partially account for their metabolic effects.[31]

Some probiotics are able to increase bacteria that express the β-fructofuranosidase and increase the beneficial short-chain fatty acid production through carbohydrate fermentation. Recent studies showed that *Akkermansia muciniphila* increases short-chain fatty acids and improves glucose/insulin homeostasis and lipid metabolism by binding to the specific G-protein–coupled receptors 41/43, enhancing glucagon-like peptide-1, and peptide YY production by enteroendocrine cells or anti-inflammatory action on immune cell production.[32] Finally, the reduction of pathobionts by probiotics decreases

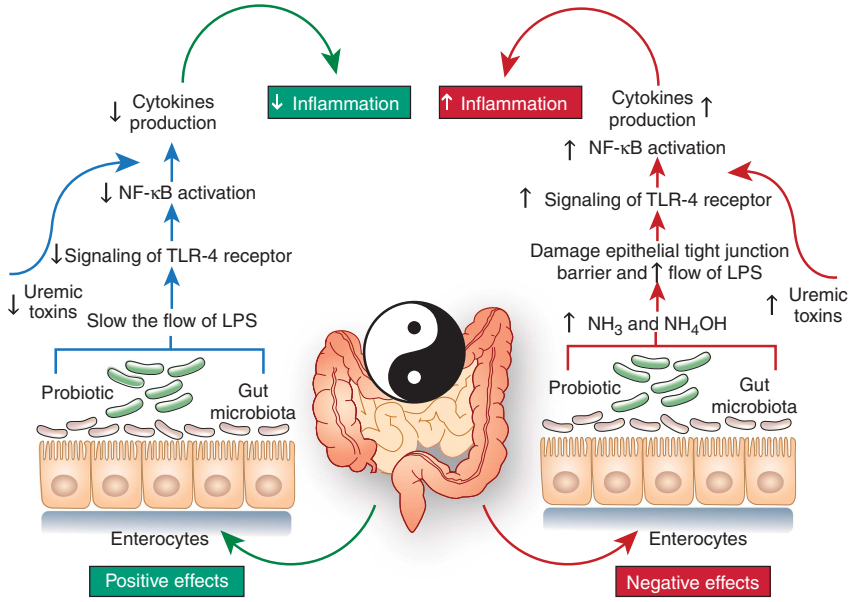

Figure 3 | The Yin-Yang aspect of probiotics. Probiotics could have ambivalent effects on the gastrointestinal tract (GIT). On one hand, probiotics could limit lipopolysaccharide (LPS) production and on the other hand, enhance the epithelial barrier, thus reducing inflammation. On the other hand, chronic kidney disease (CKD) is associated with the diffusion of a large quantity of urea in the GIT. Subsequent hydrolysis of urea by urease expressed by some probiotics and pathobionts may result in the formation of large quantities of NH_3 and NH_4OH and an increase in pH, which could affect the growth of commensal bacteria and promote the proliferation of aerobic bacteria. As a consequence, the damaged epithelial tight junction barrier by ammonia/ammonium hydroxide (NH_3/NH_4OH) and the increase in LPS flow may promote the activation of the nuclear factor-kappa *B* (NF-κB) pathway and inflammation. TLR-4, Toll-like receptor 4.

LPS production, which may in turn improve inflammation and glucose homeostasis.[29] Of note, probiotics could also help for synthesizing key vitamins such as vitamin K and B vitamins.[2]

PROBIOTICS AND CKD

To date high-quality interventional trials investigating probiotic treatment in CKD are lacking. Surrogate end points have been studied, such as changes in serum concentration or urinary excretion of biomarkers, e.g., URS or cytokines. However, studies investigating the impact of probiotics on clinical end points (e.g., CVD or mortality) have not been conducted so far. Moreover, the quality, size, and design of trials are not sufficient enough to justify the wide use of probiotics. Strict control of dietary intake as well as appropriate selection and dose of probiotic strains should be performed in order to compare trials. Experimental and clinical studies using probiotic formulations in CKD are summarized in Table 1.

The first aim of administering probiotics during CKD is URS removal. Therefore, as the production of URS, mainly generated by protein degradation, could not be completely blocked by a low-protein diet, reducing the conversion of amino acids into trimethylamine n-oxide, p-cresyl sulfate, or IS by modeling intestinal microbiota could be considered as an additional beneficial intervention.

A probiotic administration study in maintenance hemodialysis (MHD) patients reported a reduction in fecal p-cresol production, whereas plasma p-cresol decreased only slightly.[33] In two other studies performed in MHD patients, one trial detected a decrease in IS after a 5-week administration of a probiotic treatment[34] and another one a trend for a decrease in indoxyl glucuronide. In addition, the galenic formula of probiotics seems to be important. Indeed, as compared with MHD patients receiving regular capsules, only those patients treated with gastroresistant seamless probiotic-containing capsules experienced a decrease in serum IS levels.[34] It is also possible that probiotics when combined with prebiotics increase the proliferation of symbionts and probiotics. Such a compound association, called 'symbiotics', has demonstrated the ability to decrease serum p-cresol levels in nine MHD patients.[35]

To further extend the probiotics field of use, eight MHD patients were treated with oral *Lactobacillus acidophilus* for 1–6 months and showed decreased serum dimethylamine and nitrosodimethylamine, two URS associated with increased mortality in CKD.[36] To date, no data are available on potential probiotic effects on trimethylamine n-oxide generation in human. Because of the poor quality of clinical trials, it is not possible to conclude whether a probiotic supplementation may inhibit the synthesis of URS and could improve CVD. The SYNERGY study, a large double-blind, placebo-controlled, randomized cross-over study,[37] is currently under way to assess the effectiveness of symbiotics as a potential treatment aimed at reducing the synthesis of URS, specifically IS and p-cresyl sulfate.

The second aspect is whether probiotics may control chronic inflammation, where biomarkers of inflammation are inversely correlated with kidney function.[38] Andrade-Oliveira

Table 1 | Animal and human studies reporting the use of probiotics in chronic kidney disease

First author and year	Probiotics	Study	Results
Studies in CKD patients			
Viramontes-Hörner D *et al.*[42]	Synbiotic: *Lactobacillus acidophilus* and *Bifidobacterium lactis*+prebiotic (inulin)	Multicenter, double-blinded, placebo-controlled, randomized, clinical *n* = 42; HD Dietary advice (30–35 kcal/kg/day and protein 1.1–1.2 g/kg/day) Vitamins and omega-3 fatty acids supplementation 2 months	Safe Improve gastrointestinal symptoms Trend to decrease plasma C-reactive protein levels
Wang *et al.*[40]	*Bifobacterium bifidum* A218, *Bifidobacterium catenulatum* A302, *Bifidobacterium longum* A101, and *Lactobacillus plantarum* A87	Single-center, double-blind, placebo-controlled, randomized *n* = 39, peritoneal dialysis patients 6 months	↓ Serum TNF-α, IL-5, IL-6, and LPS Preservation of residual renal function
Rossi *et al.*[37]	Synbiotic: *Lactobacillus, Bifidobacteria* and *Streptococcus genera*+prebiotic (inulin, fructo-oligosaccarides, and galacto-oligosaccarides)	Single-center, double-blind, placebo-controlled, randomized cross-over trial *n* = 37; CKD stage 4–5 6 weeks, with a 4-week washout before cross-over. Dietary advice (protein 0.8 g/kg BW/d)	In process, primary outcomes: level of IS Secondary outcomes: levels of PCS; LPS, TMAO, inflammation, and oxidative stress markers; renal function; quality of life
Cruz-Mora J *et al.*[52]	Synbiotic: *Lactobacillus acidophilus* and *Bifidobacterium lactis*+prebiotic (inulin)	Single-center, double-blind, placebo-controlled *n* = 18, HD	Increases Bifidobacterial counts in fecal samples Reduction of Lactobacilli counts in fecal samples Improve gastrointestinal symptoms
Pavan *et al.* [47]	Synbiotic: prebiotic+probiotic	Prospective observation placebo-controlled, randomized trial *n* = 24; CKD stage 3–4 12 month Dietary advice (protein 0.8 g/kg BW/d)	Slowing of progression of kidney disease
Guida *et al.*[55]	Synbiotic: *Lactobacillus plantarum, Lactobacillus casei subsp. rhamnosus, Lactobacillus gasseri, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus salivarius Lactobacillus sporogenes,* and *Streptococcus thermophilus* +, prebiotic (inulin and tapioca-resistant starch)	Single-center, double-blind, placebo-controlled, randomized cross-over trial. *n* = 30; CKD stage 3–4 4 weeks	↓Plasma p-cresol
Natarajan *et al.*[41]	*Streptococcus thermophilus* KB 19, *Lactobacillus acidophilus* KB 27, and *Bifidobacterium longum* KB 31	Single-center, double-blind, placebo-controlled, randomized cross-over trial *n* = 22; HD 8 weeks	↑ Quality of life Trend in a reduction of serum indoxyl glucuronide and C-reactive protein
Miranda Alatriste *et al.*[46]	*Lactobacillus casei shirota*	Single-center, placebo-controlled, randomized trial *n* = 30; CKD stage 3–4 8 weeks	↓ Urea by 11%
Nakabayashi *et al.*[35]	Synbiotics: *Lactobacillus casei* strain *Shirota* and *Bifidobacterium breve* strain *Yakult*+prebiotic (galacto-oligosaccharides)	Single-center, observational trial *n* = 9; HD 4 weeks	↓ p-Cresol Normalization of bowel habits
Ranganathan *et al.*[45]	*Lactobacillus acidophilus* KB31, *Streptococcus thermophilus* KB27, and *Bifidobacterium longum* KB35	Multicenter, prospective, randomized, double-blind, cross-over, placebo-controlled trial *n* = 46; CKD stage 3–4 6 months	↓ BUN ↑ Quality of life
Ranganathan *et al.*[56]	*Lactobacillus acidophilus* KB31, *Streptococcus thermophilus* KB27, and *Bifidobacterium longum* KB35	Single-center, prospective, randomized, double-blind, cross-over, placebo-controlled trial *n* = 16; CKD stage 3–4 6 months	↓ BUN ↓ Uric acid concentration ↑ Quality of life
Taki *et al.*[57]	*Bifidobacterium longum*	Single-center, non randomized-placebo controlled trial *n* = 27; HD 12 weeks	↓Homocysteine, IS, and triglycerides
Takayama *et al.*[34]	*Bifidobacterium longum* strain JCM008	Single-center, non-randomized-placebo controlled trial *n* = 22; HD 5 weeks	↓ IS
Ando *et al.*[58]	*Bifidobacterium longum*	Single-center, observational trial *n* = 27; CKD patients all stages 6 monthS	Slowing of the progression of kidney disease
Hida *et al.*[33]	*Bifidobacterium infantis, Lactobacillus acidophilus, Enterococcus faecalis*	Single-center, observational trial *n* = 25;HD 4 weeks	↓ Indican in feces and in serum ↓ p-Cresol in feces
Simenhoff *et al.*[36]	*Lactobacilus acidophilus*	Single-center, observational trial One course *n* = 8; HD	↓ Dimethylamine ↓ Nitrosodimethylamine
Studies in experimental CKD			
Prakash *et al.*[44]	Genetically engineered *Escherichia coli DH5* with urease	Uremic rats (5/6 nephrectomy) 35 day	↓ Plasma urea
Ranganathan *et al.*[54]	Various combinations of probiotics	Uremic rats (5/6 nephrectomy) 16 weeks	↑ Lifespan, ↓BUN
Ranganathan *et al.*[43]	*Sporosarcina pasteurii*	Uremic rats (5/6 nephrectomy) 16 weeks	↑ Lifespan, ↓BUN
Andrade-Oliveira *et al.*[39]	*Bifidobacterium adolescentis* or *Bifidobacterium. longum*	Bilateral kidney ischemia reperfusion injury 2 weeks	↑ Acetate production Protects mice from kidney ischemia reperfusion injury

Abbreviations: BUN, blood urea nitrogen; CKD, chronic kidney disease; HD, hemodialysis; IL-5, interleukin-5; IL-6, interleukin-6; IS, indoxyl-sulfate; LPS, lipopolysaccharide; PCS, p-cresyl sulfate; TMAO, trimethylamine n-oxide; TNF-α, tumor necrosis factor-α.

et al. recently demonstrated in a mouse model of acute kidney injury that probiotic treatment increased plasma short-chain fatty acids and protected mice from kidney ischemia–reperfusion injury through modulation of inflammation.[39] In addition, a randomized, double-blind, placebo-controlled trial conducted in 21 peritoneal dialysis patients reported that a capsule containing a combination of probiotics taken daily for 6 months was effective in reducing serum levels of tumor necrosis factor-α and interleukin-6, both pro-inflammatory cytokines.[40] Natarajan *et al.* also observed a tendency for a decrease in C-reactive protein in 22 MHD patients after 8 weeks of probiotics supplementation.[41] However, the decrease in inflammatory markers was not confirmed by Viramontes-Hörner *et al.* in a study involving 2 months of symbiotic treatment in HD patients.[42] In non-dialysis patients, there is currently no study in which monitoring of inflammation was performed.

The third question is whether probiotics might improve renal function. Because of the potential beneficial effect of probiotics (reducing inflammation and uremic toxins) it is possible that renal function improves during treatment. However, studies performed in CKD only used indirect markers such as serum urea or creatinine, and no direct GFR evaluation was performed with a gold standard such as inulin or iohexol.[8] Urea and creatinine could be degraded directly by probiotics and may not reflect an improvement in renal function. Thus, the following studies should be interpreted with caution and need to be confirmed. Preliminary *in vitro* data demonstrate that *Lactobacillus delbrueckii* and *Sporosarcina pasteurii* are potential urea-targeted agents for 'enteric dialysis' and have been showed to hydrolyze urea *in vitro.*[43] Prakash *et al.* confirmed this concept by using microencapsulated genetically engineered live cells containing urease-producing *Escherichia coli*. This compound was able to reduce blood urea levels in uremic rats and reduce the conversion of urea to ammonium by bacteria.[44] Ranganathan *et al.* showed that uremic rats fed with *Bacillus pasteurii* or *Sporosarcina pasteurii* had a reduced progression of kidney disease and an extended life span.[43] To confirm this effect in humans, a double-blind, placebo-controlled, cross-over multicenter trial in 42 CKD stage III–IV patients demonstrated that the use of a probiotic cocktail was associated with a significant decrease in blood urea levels. A trend for a decrease in serum uric acid and creatinine levels was also observed.[45] Another recent study confirmed that administrating *Lactobacillus casei shirota* in 30 stage III–IV CKD patients led to a reduction in serum urea levels.[46] Furthermore, a symbiotic supplementation delayed the decline of the estimated glomerular filtration rate in 12 stage III–IV CKD.[47]

Further beneficial effects of probiotics may occur. Indeed, probiotics are able to improve constipation in CKD patients.[35] Moreover, *Oxalobacter formigenes,* which produces oxalate-degrading enzymes, might be useful for the removal of accumulated oxalates in patients with urolithiasis.[48] A majority of subjects reported having experienced a substantial improvement in their quality of life.[45]

Limitations of probiotics use should also be underlined. For example, a rise in the population of bacterial species that possess urease can increase the generation of NH_3/NH_4OH molecules, which may damage the epithelial tight junction and allow LPS to enter into the blood stream. The concomitant presence in the colic lumen of urea and urease brought by specific bacterial strains may lead to the complete loss of transepithelial electrical resistance and the near total loss of the tight junction proteins, which may favor the absorption of URS and endotoxins[49] (Figure 3). From a methodological standpoint, Hempel *et al.* noted that there was a lack of reporting of adverse events in probiotic intervention studies, and the nature of the intervention was poorly documented.[50] The available evidence in randomized controlled trials does not indicate an increased risk; however, rare adverse events are difficult to assess, and despite the substantial number of publications, the current literature is not well equipped to answer questions on the safety of probiotic interventions with confidence. In particular, probiotics are contraindicated in patients with severe immune deficiency.

FUTURE DIRECTION FOR SELECTION AND COMBINATION OF PROBIOTICS

To evaluate the capacity to survive in the GIT, *in vitro* systems using simulated or real human fluids and secretions have been developed. The ability of probiotics to adhere to intestinal epithelial cells and their antimicrobial activity have also been tested *in vitro*. These tests may represent a reliable model to predict the amount of organisms that should be delivered to the human gut by oral ingestion.[51] However, the modification of GIT medium in uremic condition (acidosis, reduced gut mobility, etc.) was not taken into account when estimating the survival of probiotics in *ex-vivo* models. Even if for most strains the amount of viable bacteria, which are able to pass through the stomach and the duodenum, is sufficient to guarantee a probiotic effect, there are some strategies such as microencapsulation with a gastroresistant material that could be used to significantly improve the effectiveness of probiotics as being demonstrated in uremic rats[44] and human.[34] In all models, the enrichment of probiotics and symbiotons in feces is a major indicator of the efficacy of probiotics. Likewise, the analysis of enzyme activities, short-chain fatty acid and endotoxin concentrations, and pH among others could be an additional tool to evaluate the probiotics activity. However, the best evidence of therapeutic benefits of any probiotic strain will be obtained from randomized, placebo-controlled trials, which are currently missing in CKD. Until now, only one study reported that a short-term symbiotic (probiotics associated with prebiotic fibers) treatment in patients with end-stage renal disease can induce an increase in *Bifidobacterium* counts.[52] Obviously, this study could not discriminate between the effects of pre- and probiotic components. Yet, this information is crucial as dysbiosis is caused by the uremic environment, which does not favor the survival of the beneficial microorganisms. Such knowledge will be required

to select future therapeutic options (e.g., using gastroresistant material, combination with prebiotics, probiotic dose).

Depending on the strain, probiotics have different underlying mechanisms of action. The rationale to use one specific probiotic strain in CKD is empirical. In other diseases, the methods used to identify potential probiotics rely on isolating different strains of *Lactobacillus, Bifidobacillu*s, or new strains identified in food and systematically testing them *in vitro* and *in vivo* in animals. The possibility that convergent mechanisms of action occur (e.g., bile salt hydrolase activity, urease, defensins, competitive exclusion behavior for uremia-generating bacteria) is currently unknown. It should also be kept in mind that probiotics may affect different regions of the intestine. Indeed, the small and large intestine have distinctive ecosystems with specific characteristics. Recently, it was proposed to identify missing bacteria and that the use of a workflow involving mouse models, clinical studies, metagenomic analyses, and mathematical modeling could help identify a probiotic candidate.[53] Thus, a better understanding of the composition of microbiota in uremic gut and its potential impact on the host will be necessary to provide an impetus in our pursuit to select the best probiotics candidate.

Moreover, organisms may behave differently when administered as a single strain versus as a combination of probiotic strains. In fact, it is widely accepted that single-cell organisms communicate to potentiate or inhibit the activity of other organisms. Multi-species probiotic preparations have been proposed to have a wide spectrum of applications, although few studies have compared their efficacy, and the differences between single or multi-stain have been inconsistent. Despite this, such an approach begins to emerge as a treatment for CKD. For example, in a rat study assessing five different probiotic combinations, only two (one containing *Bacillus pasteuri* and one *Lactobacillus sporogenes*) were able to decrease blood urea nitrogen and serum creatinine.[54]

CONCLUSION

Recent data point to the fact that dysbiosis is likely to occur in uremia given the multiple metabolic derangements. In this context, the intestinal barrier function has not yet been carefully studied. However, the fact that circulating LPS levels and bacteria-derived URS (IS, p-cresyl sulfate, trimethylamine n-oxide) increase with CKD stages suggests a link between intestinal barrier and renal dysfunction. At this point, it remains speculative but intriguing to envision that uremic microbiota and impaired GIT could account for inflammation and drive the accelerated atherogenesis and protein energy wasting in CKD. Microbial modulating therapies, in the form of probiotics, present a promising opportunity given their low cost and innocuous nature. Several experimental and clinical studies highlight that bacteriotherapy may represent an interesting approach to mitigate uremic intoxication by ingestion of live microbes that are able to catabolize URS in the gut. In addition, data demonstrate that probiotics could delay the progression of renal dysfunction and reduce inflammation markers. However, current enthusiasm for implementing the use of probiotics has been hampered, at least in part, by concerns about how precisely these various organisms mediate their beneficial effects and through the potential increase in inflammation due to hydrolysis of urea. The failure of human studies with probiotics could be explained by an unfavorable milieu that uremia creates for the symbiotic microbiota. Attempts to restore the desired microbiome by introducing favorable microorganisms without simultaneously improving the gut's biochemical milieu, by using for instance prebiotics, seem to be doomed to failure. Therefore, more basic and clinical research needs to be conducted to further understand the role of dysbiosis in the progression of CKD and its associated complications.

DISCLOSURE

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