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Current status of the microbiome in renal transplantation

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Abstract

Purpose of Review: An imbalance between pathogenic and protective microbiota characterizes *dysbiosis*. Presence of dysbiosis may affect immunity, tolerance, or disease depending on a variety of conditions. In the transplant patient population, the need for immunosuppression and widespread use of prophylactic and therapeutic antimicrobial agents creates new post-transplant microbiota communities that remain to be fully defined.

Recent Findings: Studies in mice have demonstrated significant bidirectional interactions between microbiota derived products and host immune cells. The stimulation of Treg and Th17 cells by specific products leads to maintenance of immune homeostasis versus activation of inflammation, respectively. Dysbiosis may lead to development of antigen cross-reactivity, which may affect alloreactivity. Certain immunologic sequelae of microbiota are pronounced in chronic kidney disease, due to uremia and renal metabolism of microbiota metabolites. Dietary modifications, probiotics, and fecal microbiota transplant have been investigated for alteration of microbiota in humans.

Summary: Researchers have begun to identify dysbioses associated with clinical conditions, including chronic kidney disease, post-transplant infection, and rejection. This information will allow clinicians not only to select at-risk patients for early intervention, but also to develop therapies that restore the microbiota to a state of homeostasis or tolerance..

Keywords

Microbiota; microbiome; chronic kidney disease; transplantation

Introduction

The vast community of microorganisms that exists on our epithelial surfaces is collectively termed the *microbiota*. The *microbiome* refers to the microbiota plus the surrounding environment. The microbiota is able to induce disease [1-3] and also able to regulate immunity or tolerance [4-7]. An imbalance between pathogenic and protective microbes characterizes *dysbiosis*, a perturbation in the normal composition of commensal microbiota [8]. Examples of conditions resulting from and/or contributing toward dysbiosis include *Clostridium difficile* [1], Crohn's disease [2, 9], and non-alcoholic steatohepatitis (NASH)

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[3]. In NASH, enterohepatic portal flow enables direct transfer of microbe and immune system derived inflammatory mediators from intestine to liver, leading to the activation of several molecular pathways. Henao-Mejia et al. identified that inflammasome-deficient mice had expanded *Bacteroidetes* and experienced an influx of lipopolysaccharide into the portal circulation, leading to increased hepatic tumor-necrosis factor and exacerbation of the NASH phenotype [10]. Thus, gut dysbiosis may transfer immune cells and signals to distant sites, rendering extraintestinal tissues such as organ allografts susceptible to changes in the intestinal microbiota.

In transplant patients, immunosuppression, metabolic abnormalities, and antibiotics mediate profound alterations of microbiota. This review examines this dynamic relationship at immunologic and clinical levels and presents the evidence to date describing the impact of the microbiota in renal transplantation.

Studying the Microbiome

The thousands of species and the millions of genes expressed by the microbiota (i.e. metagenome) are a reflection of the functional diversity required to maintain microbiota homeostasis. This vast array of gene expression has been studied by many groups to identify microbiome conditions that lead to health versus disease. Genetic tools to characterize microbiota include targeted sequencing of the 16S ribosomal RNA genes and shotgun sequencing of transcribed genes [11] [Table 1]. The huge amount of information obtained can be processed using principal coordinate analyses and classical multidimensional scaling [12]. Briefly, the sequenced 16s rRNA dataset is processed by phylogenetic classification to measure relative abundance of the bacterial taxa in each sample. Using extended local similarity analysis (eLSA) [13] and linear discriminant analysis effect size (LefSe) [14], one can measure differences between samples in terms of biologic diversity and biomarker expression. The Human Microbiome Project [15] and European MetaHit Consortium [16] have identified 99% of the genera present in normal human microgenomes in Western populations for investigators to use as a template for classifying individual samples or large populations.

Mechanisms of Microbiota Stimulation of the Enteric Immune System

The microbiota interacts with the intestinal epithelial cells (IEC) and resident immune cells to activate both innate and adaptive mechanisms [Figure 1]. Microbial products are sensed by receptors of the IEC, such as toll-like receptor (TLR) and nucleotide-binding oligomerization domain (NOD). For example, peptidoglycan from gram negative bacteria binds NOD1 and elicits production of CCL20 and β -defensin-3 to recruit B cells to the lamina propria (LP) and induce expression of IgA [17]. Development of intestinal lymphoid follicles depends on the presence of both microbial products and lymphoid tissue inducer cells (LTi), a subset of innate lymphoid cells (ILC), in the LP [18]. At homeostasis, bacterial products stimulate production of mucus, bactericidal molecules, and biofilms by IEC to exclude pathogenic bacteria and promote colonization by commensal bacteria [19, 20]. Virulence factors may penetrate the mucus and activate innate effectors in the LP such as natural killer T cells [21] and ILC. In response to IEC-derived pro-inflammatory cytokines,

ILC release IL-22 to help maintain the integrity of the epithelium and produce antimicrobial peptides [22]. In contrast, commensal bacteria provide tonic stimulation of the apical receptors to dampen the inflammatory response by various adaptive mechanisms. For example, *Bacteroides* and *Lactobacillus* species inhibit activation of the classical NF- κ B pathway and its downstream pro-inflammatory genes [23].

Products from microbiota engage the adaptive immune system by either directly interacting with DC or inducing IEC cytokine expression that influences recruitment and differentiation of neutrophils, macrophages, or DC [24]. At homeostasis, cytokines such as IL-33, IL-25, TGF β , and TSLP (thymic stromal lymphopoietin) activate macrophages and DC. In the presence of TGF β and retinoic acid, DC promote induced Treg (iTreg). Microbial antigens from *B.fragilis* and *Clostridia* species promote Treg development [25, 26]. Antigen-specific iTreg then migrate to lymph nodes, express anti-inflammatory cytokines such as IL-10 and TGF β , and mediate direct killing or inactivation of effector cells. In this way, iTreg suppress the immune response to commensal organisms and self-antigen at a local and systemic level.

During dysbiosis or mucosal injury, pathogenic microbial products stimulate secretion of pro-inflammatory cytokines (e.g., IL-6, IL-1, IL-23, and IL-12), which induce differentiation of CD4⁺ Th1 and Th17 cells in the small intestine [25, 26]. Th17 differentiation is induced in the gut by *Candidatus arthromitus* or segmented filamentous bacteria (SFB) [27]. Th17 cells bolster the intestinal barrier via IL-22 and prevent infection from pathogenic bacteria via IL-17 and IFN γ expression, and can thus potentially restore homeostasis in a dysbiotic environment [27].

The balance between anti-inflammatory and pro-inflammatory cells can lead to either suppression or activation of disease. The ability to alternate between pathways depends on the microbes and cytokines present. For example, retinoic acid (a cofactor for both iTreg and Th17 differentiation) is suppressed in the presence of noncommensal organisms and pro-inflammatory cytokines, thus allowing naïve T cells to be programmed into inflammatory effectors [28]. Specific antibiotic agents have been shown to block retinoic acid suppression [4]. This demonstrates how changes in microbiota may impact changes in molecular signaling that influence one T cell pathway over another.

Microbiota regulation of T cells has been associated with antigen-independent systemic inflammation. For example, intestinal SFB promote the development of rheumatoid arthritis via regulation of Th17 differentiation. This was shown in germ-free mice, where introduction of SFB alone could trigger arthritis via IL-17 expressed by Th17 cells. [4]. These data showing how a single microbe can promote a T cell subset to drive autoimmune disease demonstrate how gut microbes influence systemic inflammation.

Microbiota-derived systemic inflammation may affect alloimmunity via molecular mimicry driven by antigen cross-reactivity, such as homologous sequences between Kell antigen on red blood cells and *B.fragilis* [29]. Pantenburg et al. showed that cross reactive, primed alloreactive T cells led to accelerated skin allograft rejection in mice infected with *Leishmania major* [30]. This suggests that interactions with microbiota may alter the

immune responses to alloantigen due to the presence of cross-reactive, environmentally derived antigen.

Bidirectional relationship between microbiota and the immune system after allotransplantation

While the microbiota clearly influences immune pathways, dysregulated immune responses also impact the microbiome in a bidirectional manner. For example, absence of Treg in mice was associated with pronounced Th2 type inflammation in the gut and airways, as well as altered gut microbiota, suggesting that Treg maintain eubiosis in the gut by regulating Th2 inflammation [31]. Independent of cause, inflammation in the gut leads to changes in microbiota composition [32], leading to dysbiosis. Dysbiosis in turn disrupts the immune thresholds set by “normal” commensals, resulting in chronic, recurrent infection or inflammation and inability to clear “abnormal” microbiota [24].

Host factors in transplant patients that influence this bidirectional relationship include antibiotics, immunosuppression, and chronic kidney disease (CKD). Germ-free or antibiotic-treated mice are deficient in Th17 cells [27] and have impaired Treg function [33]. These deficiencies are associated with dysregulation of immunity, inflammation, and response to infection, leading to an increased burden of pathogens and the potential to develop cross-reactive alloantibodies. Thus, dysbiosis associated with chronic antibiotic use may have immune consequences in allograft recipients beyond simply the emergence of antibiotic-resistant organisms.

CKD is associated with several immunologic sequelae that can affect the microbiota [Table 2] [34]; conversely, the microbiota have been linked to clinical and immunologic changes in animal models of CKD [Table 3] [34-44]. The buildup of uremic toxins, fluid overload, and subsequent bowel wall edema lead to increased bacterial translocation across the gut epithelial border, causing chronic systemic inflammation [45, 46]. The microbial metabolite TMAO (trimethylamine-N-oxide) has recently been linked to increased mortality in CKD, as well as progressive renal fibrosis, platelet hyperreactivity, thrombosis, lipid metabolism, and inflammation [35-38]. These data illustrate that renal failure predisposes a host to the influx of microbial metabolites, which may have profound clinical impact on these patients.

Immunosuppression is also linked to dysbiosis. T cell depleting antibodies (anti-thymocyte globulin), non-depleting antibodies (basiliximab), and glucocorticoids may produce changes in microbiota [39]. These changes may affect one's ability to restore immunity after lymphodepletion, as compensatory homeostatic T cell proliferation is partially driven by commensal bacterial antigens [47]. While conventionally raised T cell-deficient mice show rapid T cell proliferation after adoptive transfer of naïve T cells, germ-free T cell-deficient mice undergo only a slow homeostatic proliferation [48], supporting the role of microbiota in influencing immunity in the chronically immunodeficient patient.

Clinical manifestations of microbiome disruption

Differences in microbiota after transplantation can be associated with clinically significant events. Decreased *Firmicutes* in small bowel recipients has been associated with acute rejection [49]. In lung transplant patients, restoration of microbiota diversity decreased the risk of bronchiolitis obliterans syndrome [51]. Bronchial samples containing greater than 10% *Pseudomonas aeruginosa* were associated with symptomatic infection, while those containing greater than 10% *P.fluorescens* were not. No sample had greater than 10% of both species [52]. Such changes, however, are difficult to interpret in the presence of concomitant antimicrobial administration.

Fricke et al. demonstrated that major shifts in microbiota composition (measured in blood, oral, urinary, and rectal samples) were identifiable at one month after renal transplant [53]. In another study of 26 renal transplant recipients, Lee et al. identified increased *Proteobacteria* species in rectal microbiota at 90 days [54]. Patients with post-transplant diarrhea had reduced microbiota diversity, with reduced *Bacteroides*, *Ruminococcus*, *Coprococcus*, and *Dorea*. Patients with abundant *Enterococcus* in rectal stool samples were more likely to have an *Enterococcus* urinary tract infection [54].

Changes in microbiota composition in immunosuppressed patients may also contribute to emergence of opportunistic infection. In four patients with post-renal transplant infection, there was significantly decreased *Anaerotruncus* (phylum: *Firmicutes*) in pre-transplant rectal microbiota, compared to 14 healthy control samples [53]. Absence of *Bacteroides* and *Ruminococcus* was associated with development of post-renal transplant diarrhea [54]. As mentioned above, reduced diversity of airway microbiota was associated with acute infection in lung recipients [52].

Changes in microbiota may alter metabolism of antirejection medications [55]. In a study of 19 kidney recipients who submitted rectal samples, those who required more than a 50% increase in tacrolimus dosing during the first month of transplantation were more likely to host an abundance of fecal *Faecalibacterium prausnitzii* in the first week post-transplant [55]. Oral bioavailability of tacrolimus depends partially on intestinal CYP3A4 and P glycoprotein function and is altered in renal transplant recipients with diarrhea [56]. While no causal relationship has been demonstrated, it is tempting to speculate that since gut mucosal health is associated with tacrolimus metabolism, perhaps gut microbiota contribute to drug metabolism via maintenance or disruption of mucosal integrity.

Therapeutic or diagnostic options under investigation

Characterizing microbial shifts and biomarkers associated with rejection or infection may identify patients who would benefit from earlier intervention or surveillance. This may also assist development of therapeutics that modulate the immune system to promote allograft acceptance or tolerance. Recent studies have explored the use of fecal microbiota transplant (FMT), probiotics, and dietary modifications.

Diet impacts the availability of metabolites required for intestinal immune cells. For example, retinoic acid is required for Th17 development, lymphocyte migration, and DC

maturation [57-59]. Colonocytes benefit from a fat-rich diet, and mice treated with such a diet have reduced colitis [60], inflammatory bone disease, and bone erosion [61]. These mice have altered levels of gut *Prevotella* species as well as pro-IL-1 β in neutrophils. Avoidance of certain microbe-metabolized nutrients may also help protect allograft health. In animal models, chronic dietary exposure of phosphatidylcholine, choline, or L-carnitine led to higher plasma TMAO levels in CKD subjects and was directly related to progressive renal fibrosis and cardiovascular disease [35, 38]. Although studies in renal transplant patients are limited, these observations suggest that dietary modifications may affect not only immune responses and microbiota composition, but also the availability of toxic metabolites.

Probiotics have been used in transplant recipients for the treatment of *C.difficile*. A retrospective study of lung and liver transplant recipients found *Lactobacillus* oral preparations to be safe and effective against *C.difficile* infection [62]. *Lactobacillus GG* has shown efficacy, feasibility, and safety in preventing graft versus host disease in stem cell transplant recipients [63], although prospective studies are lacking. Oral *Lactobacillus* and *Bifidobacterium* were associated with improved intestinal barrier function, partial gut microbial restoration, and reduced liver injury after liver transplant in rats [64-65]. Whether probiotics affect outcomes other than *C.difficile* infection, and which probiotics are beneficial for renal transplant recipients, is unknown.

Restoration of microbiome diversity may be feasible through FMT. This was highly effective in patients with recurrent *C.difficile* colitis [66]; however, FMT in solid organ transplant has yet to be tested. FMT for *C.difficile* colitis was not associated with significant complications (such as bacteremia) in non-immunosuppressed [66] or immunosuppressed patients [67].

Precision microbiome restoration using only a single bacterial species has recently been reported by Buffie et al. In a murine model, the investigators treated mice with different antibiotic regimens and identified distinct changes in microbiota composition and *C.difficile* susceptibility among the groups. They used a cohort of immunosuppressed, antibiotic-treated, stem-cell allotransplanted humans to identify native bacteria that displayed strong inhibition against *C.difficile* in both humans and mice. *Clostridium scindens* significantly enhanced resistance to *C.difficile* infection after adoptive transfer into mice, while transfer of other *Clostridia* species did not [68]. Recently, FMT in a kidney-heart transplant recipient with recurrent *C.difficile* was associated with restored microbiota diversity, improved clinical outcome, and loss of vancomycin-resistant enterococcus fecal dominance [69].

Certainly, avoidance of broad spectrum antibiotic use is essential to preserving microbial biodiversity and limiting development of resistance. Unfortunately, the immunosuppressed patient often requires empiric antimicrobial coverage to prevent overwhelming sepsis. Discovering ways to restore microbiota diversity or introduce essential microbial byproducts in this situation is thus highly desirable.

Conclusions

Dysbiosis in the renal transplant patient may occur as a product of several contributing circumstances, including the effects of chronic kidney disease on systemic immunity and the gut microbiome; immunosuppression regimens; exposure to alloantigen and infection; and antimicrobial therapy. Microbiota affect changes in innate molecular defense mechanisms and shape T cell populations that can shift the immune climate toward pro- or anti-inflammatory conditions. Microbiota thus represent an important point of regulation for immunomodulation in the transplant patient.

Shifts in the microbiota post-transplant have been recently identified; however, larger studies are needed to understand how microbiome alterations before and after transplantation can be used to predict clinical response to therapy, select those at risk for rejection or opportunistic infection, and design targeted therapies that promote health and graft tolerance.

Abbreviations:

C.diff	Clostridium difficile
CKD	chronic kidney disease
DAMPs	danger-associated molecular patterns
DC	dendritic cells
eLSA	extended local similarity analysis
ESRD	end stage renal disease
FMT	fecal microbiota transplant
IEC	intestinal epithelial cells
ILC	innate lymphoid cells
iTreg	induced Treg
LefSe	linear discriminant analysis effect size
LP	lamina propria
LTi	lymphoid tissue inducer cells
LPS	lipopolysaccharide
NASH	non-alcoholic steatohepatitis
NIH	National Institute of Health
NOD1	nucleotide-binding oligomerization domain 1
SDD	selective digestive decontamination
Th	T helper

TLR	toll-like receptor
Treg	regulatory T cells
iTreg	induced regulatory T cell

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* of special interest

** of outstanding interest

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Key Points

1. The microbiota interacts with the host in a bidirectional manner; microbes stimulate innate and adaptive mechanisms, and in turn require immunocompetence to suppress pathogenic bacteria and maintain gut homeostasis.
2. Gut dysbiosis may cause transfer of immune cells and signals to distant sites, rendering extra- intestinal tissues such as organ allografts susceptible to changes in the intestinal microbiota.
3. Dysbiosis may be associated with antigen cross reactivity due to molecular mimicry, potentially affecting the response to alloantigen.

Figure 1.

Commensal signals such as peptidoglycan, segmental filamentous bacteria (SFB), and polysaccharide A either directly activate gut epithelial receptors (A) or indirectly stimulate differentiation of naïve CD4 T cells via cytokines such as TGFβ and Rorγt. Presence of differentiated CD4 T cells in turn influences microbiota composition (B). Effector T cells migrate via lymphatics to regional and systemic lymphoid tissue to interact with other immune cells (C). MHC, major histocompatibility complex; TLR, toll-like receptor.

Table 1:**Methods to study microbiota**

16S rRNA gene sequencing	Distinct from eukaryotes, ribosomal RNA is used to identify bacterial and archaeal species based on hypervariable regions. Provides only phylogenetic description of microbiome.
Metagenomics	Genomic analysis of microorganisms by direct sequencing of DNA
Shotgun RNA sequencing	Identifies transcriptional products of a microbiome.
Bioinformatics: Principal coordinate analysis Classical multidimensional scaling Extended similarity analysis Linear discriminant analysis effect size	Used to visualize similarities or dissimilarities in a complex data set.

Table 2:

Immunologic changes during chronic kidney disease (Anders 2013)

Increased production of pro-inflammatory cytokines
Increased complement activation
Activation of adhesion molecule expression in endothelial cells
Increased production of reactive oxygen species inducing oxidative stress and endothelial dysfunction
Decreased phagocytic function
Impaired antigen-presentation
Impaired ratio of CD4/CD8 T cells
Impaired B cell response
Shift of Th1/Th2 ratio toward Th2 cells

Table 3:

Evidence for role of microbiota in chronic kidney disease

Tang et al. 2015, Warrior et al. 2015; Zhu et al. 2016, Tand et al. 2013	Gut microbial metabolite trimethylamine-N-oxide (TMAO) directly contributes to progressive renal fibrosis and dysfunction in animal models; increases mortality risk in CKD; enhances platelet hyperactivity and thrombosis risk; regulates lipid metabolism and inflammation; TMAO may be linked to specific dietary nutrients and gut microbes.
Wang et al. 2012	Experimental uremia in rats increases bacterial translocation from gut, which is associated with higher serum IL-6 and C-reactive protein levels. (Wang 2012)
McIntyre et al. 2011	Progressive levels of circulating bacterial endotoxin and LPS with CKD are highest in patients on dialysis. LPS levels are an independent predictor of mortality.
Anders et al. 2007	Bacterial products activate pattern-recognition receptors on various immune cells inside and outside the kidney.
Kiechl et al. 2002	Increased LPS/TLR-4 signaling may promote accelerated atherogenesis in CKD.
Ranganathan et al. 2006	Oral intake of nonpathogenic <i>Sprosarina pasteurii</i> improved renal function and survival of uremic rats.
Niwa et al. 2011	Oral neutralization of indoxyl sulfate (a bacteria-derived uremic toxin) delays progression of CKD and cardiovascular disease in uremic rats.