The Potential Role of the Gut Microbiota in Kidney Transplantation

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Introduction

Through use of high throughput sequencing technologies, numerous studies have revealed the multifaceted role of the gut microbiota in host immunity and infection, resulting in wide-ranging implications for patients with cancer and autoimmune diseases (1). The gut microbiota’s potential to influence host immunity may be of particular importance in solid organ transplant recipients, given this population’s risk of allograft rejection and increased susceptibility to infections. In this perspective article, we present recent work on the impact of the gut microbiota in kidney transplant recipients, with a focus on infectious complications and immunosuppressive drug metabolism.

Gut Microbiota in Kidney Transplant Recipients Compared to Non-Transplant Recipients

The Human Microbiome Project characterized the microbiota of 242 healthy human volunteers and found that the gut microbiota was dominated by the phylum Bacteroidetes (2). This is distinctly different from kidney transplant recipients, as evidenced by several studies. Fricke et al. evaluated the gut microbiota using rectal swabs in 61 kidney transplant recipients and reported a high proportion of the phylum Firmicutes rather than Bacteroidetes (3). Similarly, Lee et al. evaluated the gut microbiota using fecal specimens in 26 kidney transplant recipients and also found gut dominance by Firmicutes in this cohort (4). Lee et al. further noticed that the gut abundance of Proteobacteria, a phylum which includes Gram-negative bacteria such as
Escherichia coli, increased in the first couple of weeks after transplantation. Swarte et al. studied the gut microbiota in kidney transplant recipients several years after transplantation to that of healthy controls and observed that they had a significantly lower microbial diversity in the gut compared to healthy controls (5). However, none of these studies compared the kidney transplant cohort to a non-transplant, end-stage kidney disease (ESKD) cohort, so it is unclear whether this gut microbiota signature is unique to transplant recipients, or if it is pervasive in ESKD patients as well. Nevertheless, the studies do find an altered species composition and lower microbial diversity in the gut microbiota of kidney transplant recipients compared to that of healthy controls.

**Commensal Gut Microbiota and Inhibition of Pathobionts**

Recent work has shown an intricate relationship between commensal gut bacteria and pathobionts, resident microbes with pathogenic potential that are often responsible for infectious complications. Several studies have elucidated mechanisms by which commensal bacteria inhibit the growth of common pathobionts via secretion of specific metabolites. Caballero et al. studied a four bacteria consortium that included *Blautia producta* using an antibiotic-treated mouse model and reported that administering the consortium provided colonization resistance against vancomycin-resistant *Enterococcus* (VRE) (6), an organism which commonly causes nosocomial infections in the transplant population. The group further investigated the mechanism and reported that a specific strain of *Blautia producta* produces a lantibiotic antimicrobial peptide that inhibits VRE.
growth (7). Other studies have also established a role for short-chain fatty acids (SCFAs) in inhibiting the growth of Gram-negative bacteria. Sorbara et al. observed that ampicillin-treated mice had less abundance of SCFAs than controls and were more susceptible to carbapenem-resistant \textit{Klebsiella pneumoniae}. They further showed that SCFAs directly inhibit \textit{K. pneumoniae} and other common Gram-negative bacteria in the \textit{Enterobacteriaceae} family at an acidic pH similar to physiological concentrations in the human gut (8). Recent studies have also established a role for secondary bile acid formation in inhibiting \textit{Clostridioides difficile}. Buffie et al. reported that introduction of \textit{Clostridium scindens}, which converts primary bile acids to secondary bile acids via 7 alpha-dehydroxylase, provides enhanced resistance to \textit{C. difficile} infection in a mouse model (9). Taken together, these studies have elegantly established how commensal organisms may inhibit the growth of potential pathobionts in the gut.

**Gut Microbiota and Infectious Complications in Allogeneic Hematopoietic Cell Transplant Recipients**

Recent investigations in allogeneic hematopoietic stem cell transplant (HSCT) recipients show that the balance of commensal organisms and pathobionts in the gut has a significant impact on infectious outcomes. In a study of 94 HSCT patients, Taur et al. profiled serial stool specimens using 16S rRNA gene sequencing of the V4-V5 hypervariable region and found that a fecal abundance of greater than 30% of \textit{Enterococcus} was associated with a future risk for VRE bacteremia (10). A follow up study of 696 HSCT patients by this group found that a gut abundance of greater than
30% of Gram-negative bacteria was associated with future development of Gram-negative bacteremia and that fluoroquinolone prophylaxis decreased the risk for Gram-negative bacteremia (11). These studies suggest that intestinal domination with pathobionts in the gut can lead to the development of sepsis; however, they did not evaluate whether the bloodstream isolates came from the gut. A study by Tamburini et al. sought to evaluate this concept in 32 HSCT patients. Using strain level analysis, the group determined the strains in the gut prior to the development of bacteremia were most similar to the isolates from subsequent blood cultures (12). The gut microbiota may also have a role in the development of viral infections. Haak et al. investigated 360 HSCT patients and noted that a gut abundance of greater than 1% of butyrate-producing bacteria was associated with a decreased risk for the development of lower respiratory tract viral infections (13).

**Gut Microbiota and Infectious Complications in Kidney Transplant Recipients**

Similar to reports that demonstrate that the gut microbiota modulate the risk of infectious complications in HSCT patients, recent work suggests that there may also be a relationship between commensal microbiota and infections in kidney transplant recipients. Magruder et al. investigated the role of the gut microbiota in the development of urinary tract infection (UTI), which is one of the most common infections that kidney transplant recipients encounter after transplantation. In a study of 168 kidney transplant recipients, they serially profiled fecal specimens using 16S rRNA gene sequencing and found that gut abundance of *Escherichia* was independently associated with future
development of *Escherichia* UTI (14). Detailed metagenomic sequencing of paired gut and urine specimens collected in patients with UTI revealed that the *E. coli* strain in the urine was most similar to the *E. coli* strain in the gut in several cases, supporting gut bacteria as a source for UTIs. The group may have also identified protective gut bacteria; specifically, there was a trend towards significance for increased gut abundances of *Faecalibacterium* and *Romboutsia* to be associated with a decreased risk for *Enterobacteriaceae* UTI (15). Interestingly, *Faecalibacterium* and *Romboutsia* produce SCFAs, which have been shown to inhibit the growth of *Enterobacteriaceae* (8). Furthermore, the abundance of these bacteria were negatively correlated with the abundance of *Enterobacteriaceae*, supporting the concept that SCFA-producing gut bacteria may be protective of *Enterobacteriaceae* UTIs via inhibition of *Enterobacteriaceae* growth in the gut.

Further analysis of the 168 kidney transplant recipients also revealed a potential association between gut bacteria that produce butyrate, an SCFA, and protection from respiratory viral infections (16). A relative abundance greater than 1% of butyrate-producing gut bacteria was associated with decreased development of respiratory tract viral infections in the first 2 years after transplantation, consistent with data from Haak and colleagues in the HSCT population.

There is now limited data, albeit mostly in the form of case reports, which suggest efficacy of gut microbiota-based therapies, such as fecal microbial transplantation (FMT), for preventing infectious complications other than *C. difficile* infection. In one
case study, a heart and kidney transplant recipient had recurrent \textit{C. difficile} infection and also recurrent \textit{Enterococcus} bacteremia and UTIs (17). After undergoing FMT for recurrent \textit{C. difficile} infections, the patient was free not only of \textit{C. difficile} infection but also of \textit{Enterococcus} bacteremia and UTIs up to the time of publication of the case report. In a study of 8 non-transplant patients who had recurrent UTIs and received FMT for the indication of recurrent \textit{C. difficile} infections, FMT was associated with a significant decrease in UTI recurrence (18). Further studies are needed to investigate the role of gut microbiota-based therapies for preventing infectious complications in kidney transplant recipients at high risk for non-\textit{C. difficile} recurrent infections.

\textbf{Gut Microbiota and Metabolism of Immunosuppressive Medications}

The alteration of the gut microbiota due to medications, including antibiotics, has been extensively described. There is now growing interest in how the gut microbiota affects drug metabolism. Recent work by Zimmerman et al. evaluated 76 human gut bacteria and their potential ability to metabolize 271 drugs and found considerable gut microbiota-mediated drug metabolism \textit{in vitro} (19). Recent studies have specifically elucidated the role of the gut microbiota in influencing the metabolism of two of the most commonly used immunosuppressive medications in kidney transplant recipients: tacrolimus and mycophenolate mofetil.

In a pilot study of 19 kidney transplant recipients, Lee et al. evaluated the gut microbiome using 16S rRNA gene sequencing at 1 week after transplantation and
reported a correlation between higher gut abundance of *Faecalibacterium prausnitzii* and higher tacrolimus dosage at 1 month after transplantation (20). The mechanism behind this association was not initially understood. However, a follow-up study by Guo et al. found *in vitro* evidence that *F. prausnitzii* and more than 20 commensal gut bacteria directly metabolize tacrolimus into a metabolite, M1, that is 15-fold less immunosuppressive than parent tacrolimus (21). They also found that M1 is a bacterial metabolite unique to gut bacteria, as incubation of tacrolimus with hepatic microsomes did not produce M1, and that M1 production can be detected in fecal specimens obtained from kidney transplant recipients (21). The group further investigated the pharmacokinetics of M1 and parent tacrolimus after oral administration of tacrolimus in 10 kidney transplant recipients and found detectable but variable levels of M1 in the blood of all patients (22). Along with their previous finding of M1 production in the stool of kidney transplant recipients (21), the detection of M1 in the blood suggests that *in vivo* gut metabolism of tacrolimus is present. The relationship between the gut microbiota and tacrolimus is likely generalizable, as a recent study of 24 heart transplant patients also showed an association between gut microbiota diversity and tacrolimus dosing requirements (23).

The metabolism of mycophenolate mofetil, another commonly used immunosuppressive drug in the kidney transplant population, may also be influenced by the gut microbiota. Enterohepatic recirculation of mycophenolic acid, the active form of mycophenolic mofetil, has been described (24) but the mechanism has not been explored in detail. Recent work by Taylor et al. investigated the role of bacterial beta-glucuronidase, an
enzyme which can convert glucuronidated mycophenolic acid back to the active mycophenolic acid (25). The group reported that vancomycin eliminated bacteria with beta-glucuronidase activity, thereby decreasing side effects of mycophenolate mofetil such as weight loss and colonic inflammation (26). Further studies are needed to better understand the extent of the gut microbiota’s impact on mycophenolate mofetil’s metabolism and toxicity.

Conclusions

The gut microbiota is increasingly recognized as influencing a variety of complications associated with kidney transplant recipients. Its role in post-transplant infections has been recently suggested, but further studies are needed to better understand how commensal organisms function as a community to combat infectious complications. Further characterization of these microbial consortia may allow the development of defined microbiota-based treatments for the prevention of infections. In addition, some bacterial subsets have protective properties from an infection standpoint and may also directly metabolize immunosuppressive medications essential for preserving graft function in kidney transplant recipients. Further research on the dynamics between the gut microbiota and immunosuppressive drug metabolism has the potential to personalize immunosuppressive therapies for kidney transplant recipients and thereby reduce the post-transplant complications of infection and graft loss.
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