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Gut microbiome dysbiosis in the setting of solid organ transplantation: What we have gleaned from human and animal studies

Sharma A *et al*. Gut-microbiome-dysbiosis in solid organ transplantation

Abstract

The human gut microbiome refers to all of the microorganisms present throughout the length of the gastrointestinal tract. Gut flora influence host metabolic and immune processes in myriad ways. They also play an important role in maturation and modulation of the immune system. Dysbiosis or a pathologic alteration in gut flora has been implicated in a number of diseases ranging from metabolic, autoimmune and degenerative. Whether dysbiosis has similar implications in organ transplant has been the focus of a number of pre-clinical and clinical studies. Researchers have observed significant microbiome changes after solid organ transplantation in humans that have been associated with clinical outcomes such as post-transplant urinary tract infections and diarrhea. In this article, we will discuss the available data regarding pathologic alterations in gut microbiome (dysbiosis) in solid organ transplant recipients as well as some of challenges in this field. We will also discuss animal studies focusing on mouse models of transplantation that shed light on the underlying mechanisms that explain these findings.

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Core Tip: The human gut microbiome refers to all of the microorganisms present throughout the length of the gastrointestinal tract. Gut flora influence host metabolic and immune processes in myriad ways. Gut microbiota alterations have been described in solid organ recipients. In this review we discuss available human studies about changes in gut flora in solid organ transplant such as kidney, liver and small bowel.

INTRODUCTION

The human gut microbiota refers to all of the microorganisms present throughout the length of the gastrointestinal tract and include bacteria, viruses, protozoa, and fungi. The term microbiome is used to describe these microorganisms along with their collective genetic material. In this article, the terms microbiome/microbiota will be used interchangeably. We now know that there are over 100 trillion microbes in the human gut alone, with the majority being found in the colon[1].

Most of these microorganisms consist of bacteria, along with smaller numbers of viruses, fungi, and protozoa. Previous studies of gut microbiota relied heavily on culture methods and could reliably detect only a small minority of organisms. Advances in molecular technology with methods such culture independent RNA and meta-genomic sequencing have revolutionized our understanding of the composition and function of gut flora and ways they influence host metabolism, immunity and inflammation.

The importance of gut flora in maintaining a healthy physiologic state cannot be understated. Research studies have shed light on the fact that a multitude of host processes depend on microbial function. These include maintaining the integrity of gut epithelial cells and thereby the epithelial barrier, modulation of immune system[2], nutrient processing and regulating systemic inflammation and metabolism through production of chemical messengers[3,4]. One example of these messengers are short chain fatty acids (SCFAs) that are produced by bacterial fermentation of dietary fiber in the gut lumen and circulate in the bloodstream with resultant downstream organ effects[5]. Due to their enormous contribution to the host, researchers have referred to the gut microbiome as the "second human genome". Dysbiosis is defined as a pathologic alteration in the microbiota that has adverse consequences for the host. This could manifest either as bloom of pathogenic organisms, loss of commensals or loss of diversity. Both animal and human studies have described the association between dysbiosis and diseases as diverse as such as coronary artery disease, chronic kidney disease[6], liver cirrhosis, diabetes mellitus and autoimmune conditions like systemic lupus erythematosus and rheumatoid arthritis[7-9].

The advent of modern immunosuppressive drugs has revolutionized transplant outcomes in the short term due to a dramatic reduction in the incidence of acute rejection. However long-term allograft survival remains sub-optimal[10]. It has been noted that allograft outcomes vary according to the type of organ transplanted. For instance, lung and intestine grafts that are considered colonized with microorganisms have poorer graft outcomes than heart and kidney grafts (not colonized)[11]. Gut bacteria play an important role in maturation and "setting the tone" of the host immune system[2]. Given their pivotal role in shaping immunologic responses, gut microbiome can possibly affect graft outcomes in transplantation. In this review we discuss the available data regarding pathologic alterations in gut microbiome (dysbiosis) in solid organ transplant recipients. We will also explore data from preclinical studies on mouse models of transplantation that shed light on the possible mechanisms behind these findings.

METHODOLOGY

Literature search was conducted on PubMed using Mesh database for papers until March 2021. We also cite high-quality articles in Reference Citation Analysis (https://www.referencecitationanalysis.com). Only studies published in English were considered. Search terms on Mesh database consisted of "Dysbiosis", "Gut microbiome", "Kidney transplantation", "Liver transplantation", "heart transplantation", "Heart lung transplantation" and "Lung transplantation".

Organ transplantation is associated with changes in gut microbiome

Solid organ transplant recipients are exposed to a variety of factors that can affect gut flora. These include, but are not limited to, antibiotics used for treatment or prophylaxis of infections, immunosuppressive medications as well as other classes of medications such as antihypertensives. Numerous studies have shed light on gut microbiome changes in hematopoietic stem cell transplant recipients. In regards to the setting of solid organ transplantation, these studies are still limited and consist mostly of cross-sectional or longitudinal observational correlation studies.

Studies in liver transplant recipients

Bajaj *et al*[12] looked at liver transplant recipients and noted that they have increase in microbial diversity and decrease in endotoxin levels compared to pre-transplant cirrhotic levels. Pathogenic genera such as *Enterobacteriaceae* (*Escherichia*, *Shigella*, *Salmonella*) were decreased compared to baseline cirrhotic state while relative abundance of potentially beneficial commensals *Lachnospiraceae* and *Ruminococcaceae* were increased. Kato *et al*[13] looked at liver transplant patients and found that *Enterobacteriaceae, Streptococcaceae* and *Bifidobacteriaceae* were increased whereas *Enterococcaceae, Lactobacillaceae, Clostridiaceae, Ruminococcaceae,* and *Peptostreptococcaceae* were decreased in patients with allograft rejection. A study by Sun *et al*[14] showed that microbiota of cirrhotic patients awaiting liver transplant surgery was significantly different than controls, however in this study no significant difference was noted between post-transplant and control groups. A similar study showed that compared to healthy controls, liver transplantation was associated with decrease beneficial bacteria such as bifibacteria and lactobacillus and increased pathogenic bacteria such as *Enterobacteriaceae*[15].

Studies in kidney transplant recipients

The phylum bacteroides is dominant in normal humans as shown by the human microbiome project. In a study of kidney transplant recipients, Swarte *et al*[16] found that gut microbiome composition was significantly different from that of healthy controls, and had a lower diversity. Use of mycophenolate mofetil (MMF) correlated to a lower diversity of gut flora as well. Lee *et al*[17] in a study looking at 26 kidney transplant recipients found that instead of bacteroides the dominant phylum was *firmicutes*. The same group also showed significant differences in gut bacteria between kidney transplant patients that had post-transplant complications such as diarrhea, acute rejection and *Enterococcal* urinary tract infections *vs* those that did not. Similar findings were noted in pediatric kidney transplant recipients[18].

In a study of intestinal transplant patients, ileal microbial diversity as measured by Shannon indices were not different between patients with and without allograft rejection however patients with acute graft rejection had significantly higher relative abundance of *Proteobacteria* and lower abundance of *firmicutes*[19]. In a study by Yuzefpolskaya *et al*[20], stool samples of patients who had received a heart transplant within the past 6 mo showed a decrease in microbial diversity.

Metabolic changes after solid organ transplant and changes in gut microbiome: New onset diabetes after transplant

New onset Diabetes after transplant (NODAT) is a frequent complication in solid organ transplant recipients. Microbiota changes have been described in these patients that were non diabetic pre transplant. In a study of kidney transplant recipients, the relative abundance of *Akkermansia muciniphila* decreased significantly after transplant in NODAT and in initially diabetic patients but not in controls[21].

Viral infections after transplant

In a study of 168 kidney transplant recipients, Lee *et al*[22] showed that patients with high levels of butyrate producing gut (BPG) bacteria in their stool had a significantly decreased risk for development of respiratory viral infections such as rhinoviral and coronavirus infections and influenza at 6 mo, 1 year and 2 years post transplantation. It was also noted in the study that the higher BPG bacteria group had a decreased risk for development of cytomegalovirus viremia at 1 year post kidney transplantation.

The above-described studies have a number of limitations. These include small sample size and patient heterogeneity. The timing of sample collection after transplant also varied between studies. Hence the pivotal question of whether dysbiosis is merely associated with rather than directly causing post-transplant adverse outcomes remains unanswered.

Evidence from animal models of transplantation

Mice with allogenic skin grafts have been studied to understand immune processes during transplantation. It has been shown that considerable immune defects are detectable in germ-free mice that lack gut flora[23]. In these mice, smaller Peyer's patches are noted and the number of CD4^{+} T cells and immunoglobulin A producing plasma cells are found to be reduced. This highlights the important role that gut microorganisms play in maturation and development of host immunity. In a landmark study, Lei *et al*[24] found that both germ-free and antibiotic-pre-treated mice exhibit decreased allo-immunity and had increase in survival of skin grafts. This phenomenon was associated with reduction in type I interferon and nuclear factor-κB pathway activation in dendritic cells. In the same study when these germ-free mice had gastric inoculation of gut bacteria from conventional mice, accelerated skin graft rejection occurred.

Pre-clinical studies show that both innate and adaptive immune responses are affected by gut flora[25,26]. Intestinal epithelial cells express surface toll-like receptors on their surface and these are activated by binding to microbial ligands also called microbe associated molecular patterns MAMP. This binding suppresses the inflammatory response and promotes tolerance to normal microbiota components by the host immune cells. Gut flora also stimulates Treg cells which are known to play a role in graft tolerance. Depending on whether gut flora prime or quiesce the immune system of a mouse model, changes in allograft outcomes can be seen. If gut bacteria activate inflammatory pathways, this can hasten allograft rejection. On the other hand, induction of inhibitory pathways can dampen the immune response and induce tolerance. A study by Emal *et al*[27] showed that microbiome inflammation and acute kidney injury after ischemia-reperfusion *via* maturation of macrophages. Conversely, depletion of the microbes significantly attenuated renal damage, dysfunction, and remote organ injury and maintained tubular integrity after ischemia-reperfusion.

A number of chemical messengers are produced in the gut lumen by microbial activity. These include SCFAs comprising butyrate, acetate, and propionate. Butyrate has been found to induce Tregs and increase interleukin-10 production and decrease proinflammatory cytokine production by colonic macrophages[28]. In a mouse study,

antibiotics to alter gut microbiota increased rate of acute rejection of skin grafts[29]. This indicates that disruption of the gut microbiota during early life development may have persistent effects on immune regulation.

The concept of molecular mimicry

Infections occurring prior to transplant can result in several T cell receptors (TCRs) that can cross-react with donor self-peptides/allo-major histocompatibility complex. In other words, microbial antigens can mimic allo-antigens from the graft. These have the potential to generate memory T cells that can subsequently cause injury to the transplanted organ. Infections contracted after transplantation can influence ongoing allo-immunity by influencing both native and memory alloreactive T cells independently of TCR cross-reactivity. This can lead to Th1 differentiation and heralds the onset of acute rejection[30].

Therapeutic trials of modifying microbiome in a mouse model seem promising. Supplementation with the SCFAs sodium acetate or sodium butyrate decreased dysbiosis and afforded protection against allograft rejection. This protection was dependent on the G protein-coupled receptor GPR43 and T regulatory cells. This study could prompt future clinical trials exploring prebiotic and dietary modifications in solid organ transplant recipients as a means to facilitate better long-term graft survival[31].

Microbiome and immunosuppressive drugs: A bidirectional relationship

The gut microbiome can influence pharmacokinetics of immunosuppressive medications causing either activation or inactivation of the drug[32,33]. Drug elimination can also be impacted by interference in the enterohepatic circulation by de-conjugation of liver-produced drug metabolites. Studies have shown that human gut bacteria are capable of metabolizing tacrolimus and MMF, the two most commonly used medications in solid organ transplantation. Additionally, Guo *et al*[34] showed that bacterial species belonging to the *Clostridiales* order convert tacrolimus into a less active metabolite. The same research group found that *Faecalibacterium prausnitzii*, a member of the *Clostridiales* order,

was found in greater levels in the gut of 5 kidney transplant patients in need of higher tacrolimus doses. Gut microbes can also alter the expression of metabolic liver enzymes (*e.g.,* cytochrome P450s). It is a commonly seen phenomenon that diarrhea in transplant patients can elevate tacrolimus levels. This effect is thought to be related to downregulation of intestinal cytochrome P4503A4 and P-glycoprotein activity.

Discussion

Both animal and human studies conducted thus far indicate an association between gut microbiome changes and distinct clinical consequences in solid organ transplant recipients. However, association does not imply causation and further studies are needed in this direction. The complex crosstalk between gut flora and immune cells of solid organ transplant recipients needs to be better elucidated in order to develop newer and better therapeutic strategies to improve long term graft outcomes. There remain challenges in designing and executing methodologically rigorous microbiome studies including patient heterogeneity, financial cost and distinguishing between cause, effect, and coincidental association.

<u>CONCLUSION</u>

It is clear from both animal and human studies conducted thus far that gut microbiome changes are associated with distinct clinical consequences in solid organ transplant recipients. The complex crosstalk between gut flora and immune cells of solid organ transplant recipients needs to be better elucidated in order to develop newer and better therapeutic strategies to improve long term graft outcomes. There remain significant challenges in designing and executing methodologically rigorous microbiome studies due to patient heterogeneity, financial cost and distinguishing between cause, effect, and coincidental association.

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