

Microbiome alterations following solid-organ transplantation: consequences, solutions, and prevention

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Abstract: Improved long-term survival following solid-organ transplantation (SOT) has remained elusive over the past several decades, despite significant advances in early survival. The microbiome refers to the genetic material belonging to microbes that live in an ecological balance with the human host, and its importance in human health is increasingly recognized. Extensive research pertaining to the human microbiome has demonstrated that compositional changes in the microbiome can contribute to such diseases as inflammatory bowel disease, metabolic syndrome, and (recently) many of the comorbidities that develop after SOT. It is suggested that the microbiome may be an important environmental variable that could influence health outcomes after SOT. Many factors related to SOT, including end-stage organ disease, surgery, and the use of antimicrobial prophylaxis and immunosuppressive drugs, have been shown to affect microbial composition and function negatively. These alterations could compromise health outcomes after SOT through the dysregulation of important host–microbe interactions, including the modulation of local and systemic host immune function by the gut microbiome, and could contribute to morbidity and even allograft rejection. Such interventions as synbiotic therapy and fecal microbiota transplantation have the potential to prevent or reverse disruption of the microbiome related to SOT and thereby improve the longevity of transplant recipients. Although microbiome research is still a relatively new field, progress is accelerating exponentially. Future research on host–microbiome interactions in the context of SOT will facilitate the development of microbiome-directed treatments to improve patient outcomes on pre- and post-SOT.

Keywords: transplantation, immunosuppression, dysbiosis, immune system, microbiome

Plain language summary

Since the development of effective immunosuppression, major improvements have occurred in early health outcomes following solid-organ transplantation (SOT) related to medical and surgical advances. However, long-term survival rates after SOT have remained relatively constant. One recently recognized variable that may impact health outcomes after SOT is the microbiome. The microbiome refers to the community of microbes and their genes that inhabit the human body and live in an ecological balance with the host. The microbiome has been shown to play a role in many of the comorbidities that affect patients after SOT, and because the microbiome interacts with the host immune system, it may also play a role in rejection of the transplanted organ. Furthermore, patients before and after SOT are exposed to numerous drugs and procedures that may have a negative impact on the microbiome and alter its composition and function, with potential consequences for the transplant recipient. This review explores the bidirectional relationship between SOT and the microbiome and the ways in which SOT affects the microbiome and how these changes in turn shape the health of transplant patients. We also discuss proven

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and potential interventions for treating a disrupted microbiome and promising areas for future research.

Introduction

The past few decades have witnessed significant improvements in health outcomes following solid-organ transplantation (SOT).¹⁻³ Early survival rates have risen substantially, even with the increasing use of organs from high-risk or marginal donors. This is largely attributed to medical and surgical advances, including increased efficacy of immunosuppressive therapies and reduced complications from posttransplant infections.⁴ However, in spite of these improvements, the long-term trajectory of survival post-SOT has failed to improve at a comparable rate.^{1-3,5-7} While numerous factors have been examined to explain this lack of clinical progress, a potential role for the human microbiome has recently been recognized.⁸⁻¹¹ Particularly relevant to SOT is the relationship between the microbiome and the immune system, as the microbiome has been found to interact with and modulate the gut and systemic immune systems.^{9,12-14} End-stage organ disease followed by SOT can significantly perturb the human microbiome, with the potential to influence allograft and patient health adversely. This review explores the relationship between SOT and the microbiome, with a particular focus on bacteria, and discusses potential microbial-targeted interventions that could prevent detrimental alterations in the microbiome and improve outcomes for patients after SOT. We examine clinical, preclinical, and animal-based studies, since the results of animal research have the potential to inform future human studies. Animal studies allow for the specific investigation of various aspects of the SOT process in isolation (eg, the effect of individual antibiotics or immunosuppressants) in models with consistent genetic and microbial backgrounds and contain few of the confounding variables (eg, environment, diet, and medication regimes) that complicate human clinical research on the microbiome.

The human microbiome

Although the impact of commensal microorganisms on transplant health outcomes has been postulated since 1978,¹⁵ the capability for studying these microorganisms on a large scale has emerged only over the last decade. The development of inexpensive and widely accessible technologies, including next-generation DNA sequencing, highly sensitive instruments for metabolomics, and powerful computing resources for bioinformatics, has led to a rapid expansion of the field of microbiome research^{16,17} (Figure 1). With it has come an explosion of discoveries documenting the significance of the

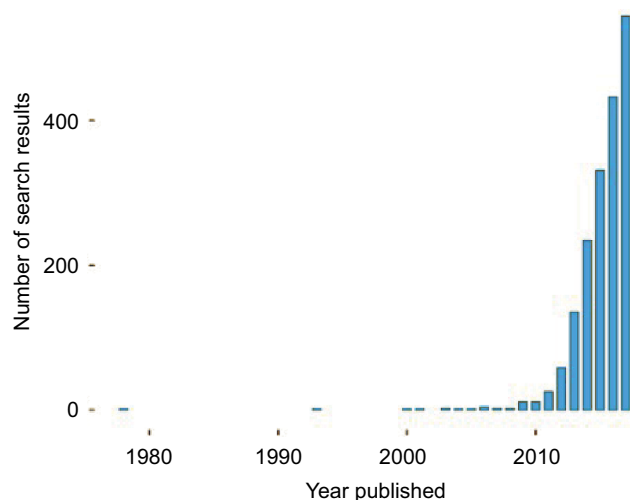


Figure 1 PubMed search results returned per year for articles relating to transplantation and the microbiome as of December 4, 2017.

Note: Search conducted using the keywords ((transplant) OR transplantation) AND microbiome.

microbiome in nearly all aspects of human health, including xenobiotic metabolism, biosynthesis of nutrients, and development and modification of the immune system.^{18,19}

The human microbiome is defined as the collection of all microorganisms, including bacteria, archaea, fungi, and viruses, that exist in the nonsterile sites of the body, including the skin, respiratory tract, and gastrointestinal tract.²⁰ The ratio of microbial to human cells is estimated to be 1.3–2.3:1, with the ratio of genetic content estimated to be an astounding 150:1.^{21,22} Microbial composition is highly variable between individuals and shaped by such factors as age, diet, medications, lifestyle, illness, and stress.²³ However, functionality appears to be quite homogeneous among individuals, indicating that the conservation of function, and not necessarily composition, may be the most important parameter for assessing changes in the microbiome (Figure 2).^{24,25} Disruption of the homeostatic state of the microbiome has been implicated in a number of diseases, many of which affect patients after SOT.^{19,26,27} Patient experiences, exposure, and treatment before and after SOT have the potential adversely to affect the microbiome and thereby influence their health outcomes.

Impact of SOT on the microbiome

Although functionality may turn out to be the most important parameter in assessing the microbiome, to date the composition of the microbiome has been studied most intensively. Shifts in microbial populations can occur in patients undergoing SOT, but high variability between transplant type and individual patients has been noted. In a study by Charlson et al,²⁸ lung-transplant recipients were found to have higher

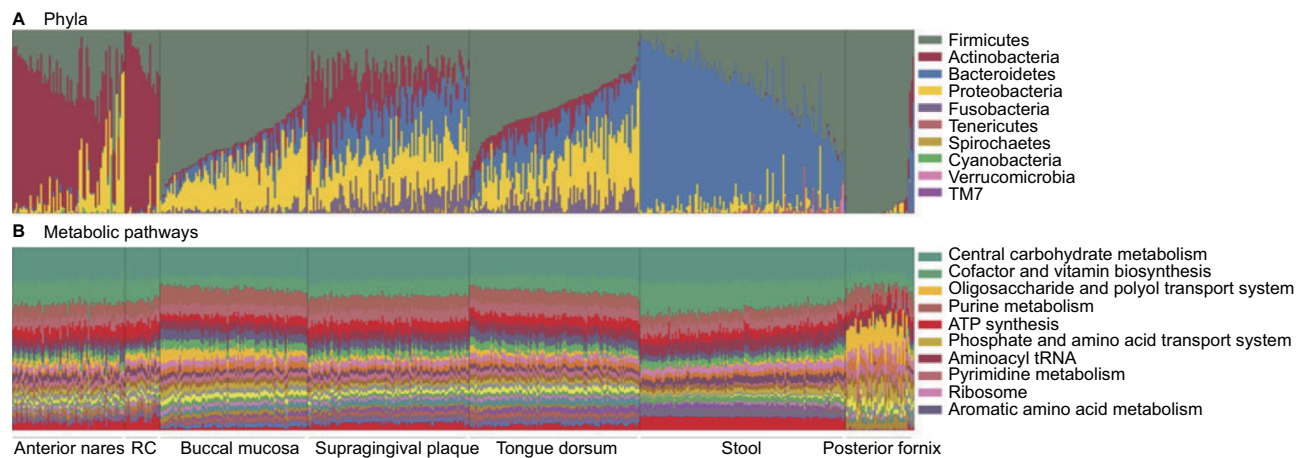


Figure 2 The human microbiome exhibits high body site (clusters along x-axis) and individual (individual columns) variation but functional diversity is highly conserved across both body site and individuals.

Notes: Height of bars denotes abundance of (A) bacterial phyla and (B) abundance of bacterial genes related to each metabolic pathway. Reprinted by permission from Springer Nature. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207–214.²⁴

Abbreviation: RC, retroauricular crease.

bacterial and fungal loads in the lower respiratory tract compared to healthy controls. Also, their bacterial and fungal communities were shown to be less diverse and/or distinct, with outlier populations present more often in patients than in nontransplant controls.²⁸ Liver-transplant patients have been found to have reduced fecal abundance of *Bifidobacterium* spp., *Lactobacillus* spp., and *Faecalibacterium prausnitzii*, but a higher abundance of Enterobacteriaceae and *Enterococcus* spp. compared to controls.²⁹ In kidney-transplant patients, taxonomic shifts in commensal bacteria have been observed 1-month posttransplant with significant interindividual variation.³⁰ Due to the high degree of variation, as well as uncertainty about what precisely comprises a healthy microbiome,²⁴ longitudinal changes in the microbiota for a specific patient may be a more reliable predictor of health outcome than interindividual comparisons.³⁰ Indeed, compositional transformations are highly variable, depending on both the type of transplant and the patient themselves, emphasizing the multifactorial nature of this issue.^{30–33} Known factors that have a role in post-SOT microbiome modifications include surgical procedures and the use of antibiotic prophylaxis and immunosuppressive drugs (ISDs).

The effect of surgical procedures on the microbiome

In addition to the effects of antibiotics, the transplantation procedure itself has been found to induce microbial alterations. Hartman et al³⁴ demonstrated that patients who underwent an ileostomy experienced a shift in the ileal microbiome from strict anaerobes (eg, Bacteroidetes and Clostridia) to

facultative anaerobes (eg, Lactobacilli and Enterobacteriaceae). This shift occurred because the ileostomy procedure allows oxygen into the small bowel, thereby encouraging the growth of organisms capable of producing ATP using aerobic respiration. This shift occurred in both transplant and nontransplant patients, suggesting it was an effect of the surgery itself, as opposed to other transplant-related factors.³⁴ Donated organs are not sterilized prior to implantation, and thus possess their own foreign microbiome. Given the inter-individual variability of the microbiome, the introduction of donor microbes could perturb the homeostasis of the recipient and possibly contribute to impaired graft function. Indeed, this complication has been demonstrated in lung-transplant recipients, where the development of primary graft dysfunction was correlated with the abundance of *Acinetobacter* in the lung microbiome of the donor.³⁵

The effect of antimicrobial prophylaxis on the microbiome

Broad-spectrum antibiotic prophylaxis may be utilized post-transplantation to prevent perioperative complications arising due to the inherent increased risk of infection from the combination of surgery and systemic immunosuppression. However, this broad-spectrum approach does not target pathogenic organisms specifically and results in off-target depletion of many beneficial commensal bacteria. For example, Hill et al³⁶ administered ampicillin, gentamicin, metronidazole, neomycin, and vancomycin, antibiotics commonly used in the perioperative period of SOT,³⁷ to mice via their drinking water, which resulted in a 10-fold decrease in total bacterial

abundance and large-scale community changes, including decreases in Firmicutes and increases in members of Bacteroidetes and Proteobacteria phyla. However, specific alterations varied with the type and duration of antibiotic treatment. It was noted that a reduction in mucosal-associated microbes in the intestine coincided with lower production of interferon gamma and interleukin (IL17A) by mucosal CD4-positive T cells, reducing the potential immunological response to infection.³⁶

Individual antibiotics can also induce large-scale microbial changes. For example, vancomycin administered to mice in the drinking water decreased the abundance of Firmicutes and Bacteroidetes,³⁸ oral ciprofloxacin reduced bacterial richness in the intestine by 30%,³⁶ and oral azithromycin exposure was found to diminish microbial richness and decrease the prevalence of Proteobacteria and Verrucomicrobia.³⁹ Altering the gut microbiome with antibiotics not only reduces microbial diversity but also impairs the metabolic potential of the microbiome. A single high dose of oral streptomycin has been found to lower the quantity of fecal microbiota and decrease levels of bacterial metabolites by 87% through effects on the metabolism of carbohydrates, fatty acids, amino acids, steroids, and eicosanoids.⁴⁰ Furthermore, an antibiotic-depleted gut microbiome can actually increase susceptibility to infection by decreasing the potential for colonization resistance by resident intestinal fauna. Colonization resistance refers to the ability of the normal gut microbiota to prevent the colonization and proliferation of microbes, usually pathogenic, that are not normally found in the gut or persist only at low densities.⁴¹ This is accomplished primarily through commensals outcompeting invading bacteria for nutrients,⁴² preventing proliferation by producing antimicrobial compounds⁴³ and triggering the host immune system to regulate bacterial growth,⁴⁴ ultimately maintaining homeostasis within the gut. Depleting the gut of resident microbes opens the door for pathogens to flourish, as is commonly observed in antibiotic-associated diarrhea. Antibiotic-associated diarrhea is caused by the proliferation of pathogenic bacteria, such as *Clostridium difficile*, a process facilitated by the antibiotic-mediated depletion of normal commensal bacteria.⁴⁵ In immunosuppressed patients, this problem is compounded by a reduced ability to clear bacterial infections, which may lead to recurrent or refractory disease.^{46–49}

The effect of ISDs on the microbiome

The increased efficacy of modern ISDs to limit graft-rejection events after SOT is a major contributor to the increase in short-term survival post-SOT.⁴ However, these ISDs have

been found to have a potentially negative influence on the microbiome.^{50–53} Turret et al⁵¹ demonstrated that prednisolone consistently reduced the ratio of Bacteroidetes to Firmicutes phyla. While composition was also altered significantly at the family level, high variability among biological replicates was noted, indicating prednisolone acted aspecifically. Tacrolimus, everolimus, and prednisolone, but not mycophenolate mofetil, resulted in decreased expression of C-type lectin, an antimicrobial peptide, in the ileum. This suggests that the relationship between the host and the gut microbiota was disrupted, which possibly contributed to the observed overgrowth of toxic *Escherichia coli*.⁵¹ Compositional changes in the microbiome of nonhuman primates, specifically in the Enterobacteriales, Clostridiales, and Lactobacillales orders and *Prevotella* and *Faecalibacterium* genera, have been found to occur after treatment with alemtuzumab, a humanized monoclonal antibody used in SOT as an induction therapy to deplete lymphocytes. The authors attributed this effect to ablation of T-cell receptor $\alpha\beta^+$ and $\gamma\delta^+$ T cells, demonstrating an association between mucosal lymphocytes and bacterial species richness in the gut.⁵⁴ In the salivary microbiome of renal and cardiac transplant patients undergoing chronic (≥ 1 year) immunosuppression using various combinations of prednisone, mycophenolate mofetil, tacrolimus, sirolimus, cyclosporine, and azathioprine, an expansion of opportunistic pathogens, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Enterococcus faecalis*, was observed.⁵⁰ This occurred despite a similar overall community structure between patients and controls, suggesting that microbial community function, drug effects, or specific bacterial subpopulations, and not simply overall composition, contributed to the permissive nature of the oral environment for pathogen colonization after SOT.⁵⁰

End-stage organ disease and the microbiome

Microbiome disruption is not only caused by the transplant surgery and essential posttransplant medications but also likely begins even before transplantation as a result of the end-stage disease that necessitated the SOT. Vaziri et al⁵⁵ found a significant difference in 190 bacterial operational taxonomic units between patients with end-stage renal disease and controls, with notable increases in the Actinobacteria, Proteobacteria, and Firmicutes phyla in patients with severe kidney disease. This effect was attributed mostly to the influx of urea, uric acid, and oxalate into the colon in these uremic patients, which altered the biochemical environment of the colon, as well as disrupting the colonic epithelial tight junc-

tions.^{55,56} In patients with liver cirrhosis, there are significant decreases in the ratio of normal commensal to potentially pathogenic bacteria in the gut compared to controls.⁵⁷ In a separate study, compositional differences characterized by decreased species richness and reduced abundance of Proteobacteria and Fusobacteria in cirrhotic patients were also seen.⁵⁸ Functionally, cirrhotic patients' bacterial metagenomes were enriched for membrane transport orthologues, and an increase in pathway modules responsible for ammonia production was also seen, suggesting that bacteria play a role in the hyperammonemia complicating liver cirrhosis.⁵⁸

Impact of the microbiome on SOT patients

It is important to note that the relationship between transplantation and the microbiome is not unidirectional but is instead bidirectional, complex, and constantly evolving. Therefore, altering a transplant patient's microbiome composition and function will potentially modify how the microbiome influences the host (Figure 3). The microbial disruptions occurring from SOT which have been described thus far, particularly in the gut, are predicted to exert a negative influence on the host. Changes in community structure and function can impair the ability of the gut microbiome to produce nutrients for the host,⁵⁹ protect the host from colonization by pathogens,²⁸ and induce protective inflammation.^{60,61} These factors can increase the risk of immune-related complications post-SOT, an idea supported by the observed correlation between microbial disruption and rejection events.^{62–64}

Bacterial dysbiosis was correlated with the rejection of small-bowel allografts in a study by Oh et al,⁶² which found that the abundance of ileal Firmicutes was lower in transplant patients experiencing rejection, whereas the abundance of Proteobacteria was higher. As well, the prevalence of

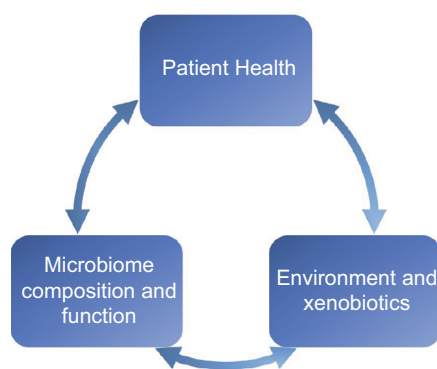


Figure 3 Bidirectional relationships between the environment and xenobiotics (including diet, lifestyle, and medications) and host microbiome and patient health. **Note:** Disruption of one variable likely to impact the others.

Escherichia and *Klebsiella* spp. was comparable in pre-rejection and active rejection samples but was distinct from samples obtained from patients with no evidence of rejection. This study reported no significant changes in the microbiome of small-bowel-transplant patients who were administered antibiotics compared with those who were not, and thus these microbiome alterations appeared to result from factors other than antibiotic treatment.⁶² In lung-transplant patients, alterations to pretransplant flora have been linked with the onset of bronchiolitis obliterans syndrome, one of the main causes of allograft loss after lung transplantation.⁶⁵ Recolonization of the lung microbiome by resident pre-SOT bacteria, specifically the reestablishment of certain *Pseudomonas* spp., is protective for the development of bronchiolitis obliterans syndrome.^{63,66} Moreover, lung-transplant patients colonized with *Simkania negevensis* in their bronchoalveolar lavage microbiome have been found to be 3.4-fold more likely to develop allograft rejection. The presence of *S. negevensis* preceded allograft rejection in 37% of lung-transplant patients, which suggests a potential causal relationship between this bacterium and rejection events.⁶⁴

While microbial dysbiosis influences rejection via many different mechanisms, the most significant may be the triggering of local and systemic inflammation.^{67,68} The observation that organs with a lower bacterial load, namely the heart and kidney, tend to have lower rejection rates than organs with higher exposure to commensal microorganisms and greater exposure to the external environment (skin, intestines, and lungs)⁶⁹ potentially supports this hypothesis.^{62–64,70–73}

Microbial-mediated immune-cell abnormalities posttransplantation

The goal of immunosuppression after SOT is the downregulation of adaptive immunity to prevent allorejection, but this can be counteracted by the priming of recipient T cells by gut microbes. In a mouse skin-graft model, both the bacterial pathogen *Listeria monocytogenes* and the commensal bacteria *Staphylococcus aureus* have the ability to stimulate and activate alloreactive T cells in a specific-pathogen-free mouse skin-graft model via the activation of antigen-presenting cells and the production of IL6, respectively.^{73,74} Importantly, these mechanisms of activation occur systemically, not only locally to the colonized tissue, suggesting that the gut microbiome is able to affect alloimmunity of distant, sterile, transplanted organs. This view is supported by a study from Lei et al,⁷⁵ where it was demonstrated that pretreating specific-pathogen-free mice with broad-spectrum antibiotics (gentamicin, kanamycin, colistin, metronidazole, and vancomycin) via

oral gavage reduced the potential for T-cell priming, as indicated by delayed rejection events of both skin and cardiac allografts.⁷⁵ The eukaryotic parasite *Leishmania major* was also found to expedite allograft rejection via priming of cross-reactive T cells, despite the administration of anti-CD40 ligand antibody as costimulatory blockade, showing this phenomenon is not restricted to bacteria.⁷⁶ Spleen cells collected from these mice were found to produce interferon gamma at a significantly higher rate than controls, indicating a high level of immunoactivity.⁷⁶ Interestingly, the activation of the adaptive immune system by T-cell priming occurs mostly through Toll-like receptor (TLR) signaling and subsequent activation of the MyD88 pathway.

Evidence demonstrates that TLR signaling plays an important role in immune system activation after SOT. TLR signaling through the MyD88 pathway has been linked with increased allograft rejection.^{72,73,77} The lower incidence of allograft rejection in individuals with variants in TLR4 further suggests that activation of this receptor is involved in allorecognition.⁷¹ Alterations in the gut microbiome can also activate the TLR4-signaling pathway, which stimulates an innate inflammatory immune response and dysregulates the intestinal epithelial barrier, leading to increased inflammation.⁷⁰ TLR4 signaling has also been found to dysregulate the gut microbiome through the altered expression of antimicrobial peptides, resulting in a feedback loop of gut microbial modulation.⁷⁸

Regulatory T cells (T_{regs}) are immunosuppressive T cells that function to regulate the activity and proliferation of effector T cells and prevent autoimmune activity through the maintenance of tolerance to self-antigens.⁷⁹ Researchers have established that antigens derived from certain microbial strains can impact the development of T_{regs} so as to promote tissue injury and alloreactive responses.⁸⁰ Microbial antigens can suppress retinoic acid, thereby hindering the development of induced T_{regs} (iT_{regs}),⁸¹ as retinoic acid is required by these cells for TGF β signaling.⁸² Since iT_{regs} can counteract tissue injury by inhibiting mucosal T_H2 responses,⁸³ the inability of iT_{regs} to develop may contribute to allograft rejection. Brandl et al⁸⁴ also demonstrated that dysbiosis induced by oral administration of vancomycin can modulate T_{reg} phenotype to increase the likelihood of proinflammatory alloreactive T-cell responses, which demonstrates additional unintended complications of posttransplant antibiotics.

Immunoactivation by bacteria can also be organ specific. In liver transplantation, the release of microbe-associated molecular patterns, such as flagellin, into the portal venous circulation was found to shape immunoactivity by influencing

the expression of major histocompatibility complex II expression (increased) and phagocytic activity (decreased) of Kupffer cells, which are liver-specific macrophages. This Kupffer cell phenotype has the potential to lead to increased risk of reperfusion injury and reduced bacterial clearance from the liver, compromising health outcomes after transplantation.⁸⁵

Additional consequences of dysbiosis on posttransplant outcomes

Allorejection is not the only adverse event caused by microbial dysbiosis that may be hindering long-term survival post-SOT. Lu et al³² found that patients who experienced significant changes in intestinal flora following liver transplantation, including shifts toward more pathogenic bacteria and a loss in overall microbial diversity, had longer hospital stays and more severe infections. These microbial imbalances were largely attributed to prophylactic antibiotic administration during transplantation.³² Loss of microbial diversity following transplantation has also been correlated with posttransplant diarrhea related to decreased abundance of Bacteroidetes, *Ruminococcus* spp., *Coprococcus* spp., and *Dorea* spp.⁸⁶

Drug metabolism research suggests that microbial changes post-SOT can also alter the impact of host metabolism on therapeutics,⁸⁷ and these effects also extend to the ISDs, including tacrolimus. Patients with a greater quantity of fecal *Faecalibacterium prausnitzii* required higher doses of tacrolimus to obtain the same therapeutic effect as patients without dysbiosis.⁸⁸ Optimizing a patient's immunosuppression is a great challenge, and the differential metabolism of ISDs due to varying microbial composition further complicates this important issue.

Solutions and prevention

Many precautions for protecting the microbiome are already standard practice in SOT, albeit mostly inadvertently. The majority of these safeguards are aimed at preventing pathological bacterial infections. However, as previously discussed, while antibiotics serve to remove harmful pathogens and may even help promote graft survival through the elimination of pathobionts,⁷⁵ the often untargeted nature of antibiotic usage may deplete commensal bacteria and produce a less diverse microbial environment that is then primed for pathogen colonization.⁸⁹ However, the outcome of antibiotic prophylaxis for an individual patient likely depends on the original or current composition of their microbiome and myriad other factors, not all of which are possible to control.

A relatively new but increasingly considered treatment is synbiotic therapy, the concurrent administration of prebiotics and probiotics.⁹⁰ Prebiotics are compounds with the potential to promote growth of healthy gut bacteria, whereas probiotics are live bacteria thought to be beneficial to health.⁹¹ While still quite broad in nature, synbiotic therapy aims to increase the diversity of the microbiome and restore proper function, as opposed to antibiotic therapy, which generally decreases diversity and impairs function. Synbiotic therapy following liver transplantation, specifically the administration of live *Bifidobacterium breve* and *Lactobacillus casei* bacteria along with indigestible galacto-oligosaccharides, reduced systemic inflammation 6-fold and decreased the rate of bacterial infections posttransplantation in a study of 50 patients randomized to receive either symbiotic therapy (2 days preoperatively and 2 weeks postoperatively) or not.⁹²

Even more targeted approaches may also be beneficial, such as supplementation with microbial metabolic products that are downregulated in a damaged microbiome. Best known are the short-chain fatty acids (SCFAs) which are end products of bacterial fermentation in the gut, and by acting to reduce inflammation play an essential role in gut health.⁹³ In patients with severe liver cirrhosis, Bajaj et al⁵⁷ identified decreases in the abundance of beneficial taxa, such as Lachnospiraceae, Ruminococcaceae, and Clostridiales, bacteria known to produce SCFAs and also compete with pathogenic bacteria for nutrients.^{57,94} Therefore, a reduction in these bacteria could lead to more frequent pathogenic infections. Restoring proper levels of SCFAs in the gut through replacement therapy could potentially compensate for the reduction in SCFA-producing bacteria and restore an appropriate functional and metabolic balance. A host of other bacterial metabolites also play an important role in maintaining homeostasis between the intestinal microbiome and the immune system. Bacterial indoles produced from the metabolism of tryptophan are responsible for stimulating IL22 production by innate lymphoid cells,⁹⁵ which in turn stimulates the production of mitogenic factors and antimicrobial peptides, promoting a healthy intestinal epithelial layer and regulation of the adjacent bacterial communities.^{96,97} Bacteria-derived polysaccharide A has been found to stimulate the production of T_{reg} cells that release IL10, limiting IL17-induced inflammation.⁹⁸ Also, the stimulation of NLRP6 by bacteria-derived metabolites taurine, histamine, and spermine maintains proper function of colon-derived inflammasomes, subsequent antimicrobial peptide production, and microbiome homeostasis.⁹⁹ Supplementing a dysbiotic intestinal environment with such bacteria-derived metabolites as these may be a feasible method of controlling inflammation

and aberrant bacterial growth, possibly helping to restore a structurally and functionally homeostatic microbiome.

Nonmicrobial interventions may also play a role in protecting the microbiome post-SOT. An inadvertent precaution taken against microbial dysbiosis is ischemic preconditioning, alternating periods of ischemia and reperfusion, performed to condition transplanted tissues to periods of restricted blood flow prior to organ procurement.¹⁰⁰ Ischemic preconditioning prior to liver transplantation has been shown to restore pretransplant microbial composition, increase species diversity and richness, and decrease hepatic graft injury.¹⁰¹

As researchers gain further insight into the influence of the microbiome on post-SOT health outcomes, it will be increasingly possible to develop targeted approaches to prevent microbial dysbiosis. One such approach may be the use of fecal microbiota transplantation (FMT) to restore the microbiome after the perturbations experienced before and during SOT. FMT has already proven to be highly successful in treating recurrent *C. difficile*, an infection attributed to microbiome dysbiosis.¹⁰² Given that FMT has been increasingly considered as a treatment in other diseases involving microbial dysbiosis (eg, metabolic syndrome and inflammatory bowel disease),¹⁰³ it may be fruitful to investigate the use of FMT to improve patient health post-SOT. Indeed, FMT has been utilized successfully in treating recurrent *C. difficile* infection in a pediatric patient (Figure 4).⁴⁹ Early recognition of the potential need for SOT could allow for the collection and storage of patients' own stool samples while their microbiome is still relatively stable, allowing for autologous FMT,¹⁰⁴ or if their dysbiosis is advanced due to their end-stage disease (see previous section), then a healthy donor could be identified.

Although there are many potential interventions to restore a dysfunctional microbiome, preventive measures to avoid microbial dysbiosis in the first place would be ideal. Limiting the unnecessary use of broad-spectrum antibiotics is feasible today, but in the future, profiling a patient's microbiome prior to a planned intervention or initiation of a drug may be useful. Knowledge regarding the composition and function of a patient's microbiome could inform the transplant team about the potential risk for infection, rejection, unexpected medication metabolism, and other potential complications as a component of personalized transplant medicine.⁶¹

Limitations of microbiome research

Despite the explosion of knowledge regarding the human microbiome over the last decade, our knowledge in the field is far from complete, due in part to many technological limitations. These include issues with DNA-sequencing

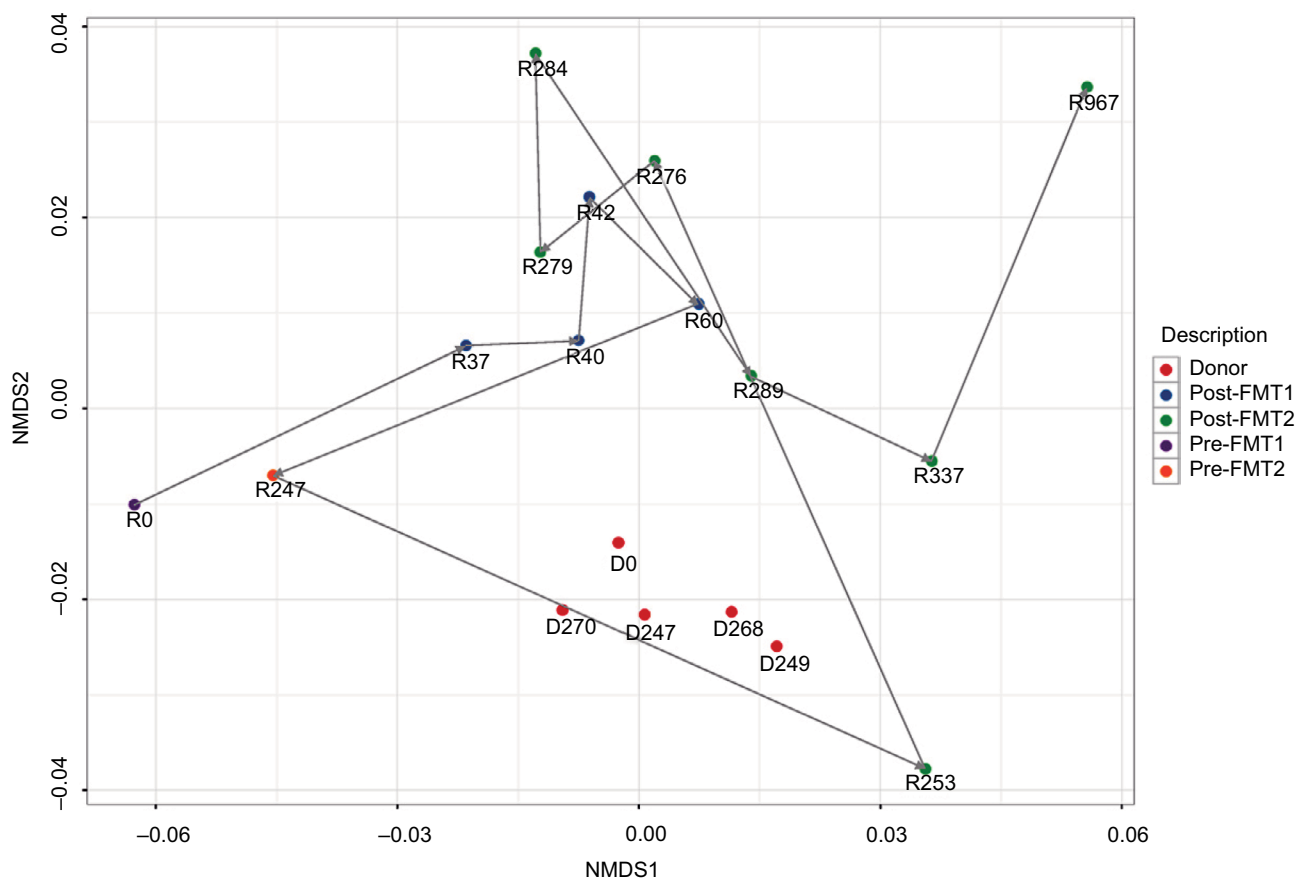


Figure 4 Changes in bacterial composition (β -diversity) before and after two fecal microbiota transplants (FMTs) were used to treat refractory *Clostridium difficile* infections in a pediatric heart-transplant patient.

Notes: Clustered red circles indicate the stable donor microbiome. The purple circle represents the sample obtained before the first FMT, followed by blue circles (post-FMT1). The patient relapsed (orange circle) after a course of antibiotics and was then treated with a second FMT. Following the second FMT, although the *C. difficile* infection did not recur, the microbiome demonstrated dynamic changes that may in part have been related to the use of mycophenolate mofetil. Reproduced from Flannigan KL, Rajbar T, Moffat A, et al. Changes in composition of the gut bacterial microbiome after fecal microbiota transplantation for recurrent *Clostridium difficile* infection in a pediatric heart transplant patient. *Front Cardiovasc Med.* 2017;4:17. Creative Commons license and disclaimer available from: <http://creativecommons.org/licenses/by/4.0/legalcode>⁴⁹

techniques,¹⁰⁵ a lack of understanding of the connections between microbial composition, genomic content, and metabolic function,²⁴ as well as confounding factors associated with human disease and the use of animals to model human diseases.¹⁰⁶ Moreover, a lack of consistency or agreement upon which techniques are best to use for microbiome studies produces high interstudy variability and confounds comparisons.¹⁰⁷ Ultimately, more time is required to resolve these issues and for significant progress to be made in the field. However, the potential for treating the microbiome as a component of transplant medicine is significant and may be key to improving patient health and prolonging survival.

Conclusion

Collected knowledge from human and animal studies and relatively small trials suggest that the human microbiome may be a significant and previously overlooked environmental factor impacting the long-term health outcomes of transplant recipients. SOT has been shown to

modulate both the compositional structure and the function of the human microbiome, and in turn, alterations in the microbiota can contribute to allograft rejection and the development of post-SOT comorbidities. While few proven interventions currently exist for treating a dysbiotic microbiome, research in the field is accelerating. At present, recognition that SOT has the potential to disrupt the important homeostasis that exists between the human host and their microbiome and efforts to minimize or correct these disruptions may be helpful in optimizing patient and allograft health.

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Disclosure

The authors report no conflicts of interest in this work.

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