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Impact of the Microbiota on Solid Organ Transplant Rejection

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Abstract

Purpose of the review: The microbiota in mammalian hosts can affect maturation and function of the immune system and has been associated with health and disease. We will review new findings on how this dynamic environmental factor impacts alloimmunity and therapy in transplant hosts.

Recent findings: The microbiota changes after transplantation and immunosuppressive therapy. New data indicate that different microbial community structures have distinct impact on graft outcome, from promoting, to inhibiting or being neutral to transplant survival. In addition, we will address reciprocal interactions between the microbiota and immunosuppressive drugs, as well as the suitability of the microbiota as a predictive biomarker and its utility as adjunct therapy in transplantation.

Summary: Advances in microbiome sequencing and wider availability of gnotobiotic facilities are enabling mechanistic investigations into the commensal communities and pathways that modulate allograft outcome, responsiveness to immunosuppression and side effects of drugs. A better understanding of the functions of the microbiota may help mitigate drug toxicity, predict drug dosage and dampen alloimmunity in transplant patients.

Keywords

microbiota; transplantation; rejection; commensals

Introduction

The microbiota, the communities of microorganisms that colonize mucosal and epithelial surfaces in contact with the outside world, lives in symbiosis with its host. Hosts become colonized at birth, with microbial diversity increasing in the first years of life [1]. Each individual carries their own microbial community structures, shaped by birth mode, diet, environmental exposures, host genetics and immune constraints [2]. In turn, the microbiota is essential for health. For instance, the gut microbiota synthesizes essential metabolites such as vitamin K, breaks down otherwise indigestible dietary plant fibers, inactivates toxic substances, and prevents pathogen colonization. The skin microbiota helps prevent cutaneous infections and accelerates wound healing.

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We will discuss four ways by the intestinal and extra-intestinal microbiota and transplantation intersect (Figure 1). First, the microbiota modulates alloreactivity. Indeed, although the strength of the alloimmune response is primarily determined by the extent of genetic disparities between the host and donor, the microbiota also modifies this response, potentially enhancing or dampening alloimmunity. Systemic effects of gut microbiota and local effects dictated by the transplanted organ-specific microbiota both contribute to this effect, perhaps explaining in part why colonized organs like the lung have worse outcomes after transplantation than non-colonized organs like the heart or kidney. Second, the gut microbiota may also participate in the metabolism of immunosuppressive drugs and consequently may affect their dosing, or cause some of their side-effects. Third, because of its dynamic status and rapid response to environmental changes, the gut microbiota might become useful as a predictive biomarker, for instance for allograft outcome, responsiveness to immunosuppression or post-transplant complications. Finally, the microbiota might be harnessed therapeutically.

Impact of the microbiota on the immune system

The hypothesis that the intestinal microbiota might modulate alloimmunity derives from observations outside the transplant field that the microbiota matures and shapes the immune system. For instance, colonization of germ-free (GF) mice with the bacterium *Bacteroides fragilis*, promoted lymphoid organogenesis and corrected immune deficits [3]. Along the same lines, mice treated with antibiotics and GF mice had a reduced number of lacteals, lymphatic-like structures that originate from each intestinal villus and help absorb dietary lipids, whereas conventionalization of GF mice restored lacteal maturation and integrity [4]. The intestinal microbiota also directs the differentiation of innate and adaptive immune cells in the gut and lamina propria. Bacterial species colonize specific niches in the gut, creating microenvironments and providing signals that dictate dendritic cell (DC) licensing to orchestrate highly specialized T cell responses. For example, segmented-filamentous bacterium (SFB) attaches to the lining of the ileum and promoted T-cell dependent IgA responses [5], Th17 [6,7] and Th1 [6] differentiation. On the other hand, intestinal *Clostridial* consortia [8,9] and *Bacteroides fragilis* [10] promoted the induction of T regulatory cells (Tregs), whereas *Bacteroides thetaiotaomicron* drove both Th1 and Treg differentiation [11]. The gut microbiota can also modulate the immune system systemically, impacting immune responses distal to the gut. For example, Abt et al. [12] showed reduced antiviral responses in mice upon oral antibiotic treatment that affected the gut microbiota, resulting in impaired viral clearance and enhanced viral susceptibility. However, it should be noted that antibiotic treatment impacts not only gut communities, but also microbiota at other compartments including the skin [13]. Colonization of GF mice with gut-tropic commensals that cannot colonize other compartments may more conclusively address whether gut commensals can have systemic immune effects. Indeed, compared with uncolonized mice, SFB mono-colonization of GF mice resulted in enhanced pathological score and proinflammatory T cell induction in the brain in an experimental mouse model of autoimmune encephalomyelitis [14], and also in exacerbated joint thickening and auto-antibody production in a rheumatoid arthritis mouse model [15]. Outstanding questions remain as to how gut-resident bacteria can impact immune responses at locations distal to

the gut. Possible mechanisms include production of microbial products or metabolites that circulate systemically, or induction of signaling in gut epithelial cells or intestinal immune cells that then either themselves travel systemically, or relay these signals to other cells. The intestinal microbiota's ability to modulate immunity in distal, sterile sites like the brain and joints suggests that the gut microbiota may affect transplantation by altering alloimmunity to non-intestinal allografts, whether colonized or sterile, as suggested by the ability of oral antibiotics to prolong survival of both skin and cardiac allografts in mice [13].

The extra-intestinal microbiota also has mutualistic relationships with the immune system. In the skin, adaptive immunity modulates tissue homeostasis and ensures that microbes do not disseminate into draining lymph nodes (LNs) [16]. Tolerance to commensal microbes in the skin is modulated in part by a large and diverse population of FoxP3⁺ Tregs, many of which are specific for microbial-derived antigens [17]. This Treg population is initially established during neonatal life when commensals colonizing body surfaces after birth enter developing hair follicles [18]. The presence of microbes on neonatal skin seems to preferentially affect Tregs over other T cell types, as GF neonates generate fewer cutaneous Tregs than SPF mice but show no differences in CD4⁺ effector, CD8⁺, dendritic epidermal, or $\gamma\delta$ T cell counts [18]. This neonatal wave of Tregs appears to be important for maintaining commensal tolerance throughout adult life. For example, mice colonized with the common human skin commensal *Staphylococcus epidermidis* (*S. epi*) as adults and those colonized from birth both achieved a state of commensalism characterized by a lack of tissue inflammation and a persistence of the microbe on the skin. However, following a minor skin-abrasion challenge, adult-colonized mice managed local invasion by the microbe more poorly than neonatal-colonized animals, exhibiting increased tissue inflammation, a larger *S. epi*-antigen-specific CD4⁺ Treg:Teff ratio in the skin-draining LNs, and augmented neutrophil infiltration of the damage site [18]. In addition to modulating commensal tolerance, the skin microbiota also modifies tissue-specific response to injury. Microbial communities on the skin promoted cutaneous accumulation of IL-17A-producing CD8⁺ T cells at steady state through a process that depended on migratory DCs and non-classical H2-M3-mediated antigen presentation [19–21]. Furthermore, tissue-resident memory CD4⁺ and CD8⁺ T cells generated by homeostatic encounters with microbes like *S. epi* or *Candida albicans* expressed paradoxical phenotypes characterized by simultaneous expression of Type 2 and Type 17 transcription factor mRNA [20]. These programs were differentially activated or suppressed in response to site-specific cues like tissue injury, allowing the organ to rapidly tune local immunity, including T cell recruitment to the wound site [19,20]. Importantly, T cells with these paradoxical programs were localized to the skin and not observed in the LNs or spleen [21], suggesting that constant, localized exposure to specific microbial communities modulates these dynamic phenotypes. Similarly, the lung microbiota has been associated with production of IL-17B and progression to pulmonary fibrosis in a bleomycin-induced fibrosis mouse model [22]. Together, these data support the hypothesis that the microbiota in transplanted colonized organs may also affect local alloimmunity and graft survival by interacting with local immune cells.

The impact of the microbiota on alloimmunity and transplant outcome

The microbiota at various body sites changes after transplantation [23], consistent with the use of peri-operative and prophylactic anti-microbials. In addition, taxonomic changes in the microbiome have been associated with acute or chronic rejection [24–27]. Because it is difficult to prove causality in humans, we used antibiotic-treated and GF mice to determine whether the microbiota causally modulates alloimmunity and graft outcome. Oral antibiotic pre-treatment of donors and recipients prior to transplantation, or a GF status, both prolonged survival of minor and major mismatch skin grafts, and of MHC class II-mismatched heart allografts [13], and reduced chronic pathology in minor mismatch lung allografts [28]. Conventionalization of GF mice with fecal microbiome transfer (FMT) from control colonized mice but not from antibiotic-treated mice restored accelerated rejection of minor mismatch skin grafts by GF mice [13]. This demonstrated sufficiency of the microbiota in accelerating transplant rejection, and that different microbial community structures can affect transplant outcome differently. Prolonged survival of the minor mismatch skin graft in antibiotic-treated and GF mice was due to reduced licensing of LN DCs that displayed diminished expression of genes involved in NF- κ B and type I IFN signaling, and had lower ability to prime donor-reactive T cells in the graft-draining LNs [13]. Indeed, mice deficient in IFN α R displayed prolonged minor mismatched skin graft survival that was not further extended by antibiotic pre-treatment.

These results demonstrate that certain community structures can augment alloimmunity and accelerate rejection while others are neutral (those remaining after antibiotic treatment). There have been other reports indicating that yet other communities can dampen transplant rejection, an observation that supports manipulating the microbiota as an adjunct therapy to improve transplant outcomes. For instance, Rey and colleagues have shown that antibiotic treatment early in life, which prevents normal taxa acquisition, accelerated rejection of aortic allografts [29], suggesting that mice acquire protective bacteria after weaning. Consistent with some microbial communities being protective, we found that mice obtained from Jackson displayed longer survival of minor mismatch skin grafts than genetically similar mice obtained from Taconic Farms but colonized with different microbiota [30]. Interestingly, co-housing or FMT dominantly converted fast rejectors into slow rejectors, a phenomenon associated with the fecal presence of an *Alistipes* genus in slow rejectors [30]. Guo and colleagues have also implicated the microbiota in the differences in acute and chronic rejection of minor mismatch lung allografts observed in mice purchased from different vendors [31]. This outcome divergence was associated with different proportions of lung Tregs. Importantly, antibiotic pre-treatment of the spontaneously tolerant mice reduced lung Tregs and restored the acute and chronic rejection phenotype, again suggesting protective microbiota. These two studies also emphasize the importance of the microbiota in determining experimental variability in transplantation, as described in other settings [32]. Microbiota protective for transplantation can also be induced therapeutically or through tolerogenic states like pregnancy. Indeed, Zhang and colleagues demonstrated that FMT from untransplanted mice that had received high-dose tacrolimus improved full mismatch skin graft survival in recipients treated with a low-dose tacrolimus [33], suggesting the presence of protective microbes in high-dose tacrolimus fecal samples. Bromberg and

colleagues reported that FMT from pregnant females improved survival of full mismatch heart allografts in animals treated with low-dose tacrolimus, which correlated with the presence of *Bifidobacterium pseudolongum* [34]. Notably, the administration of this bacterial strain to transplant recipients was sufficient to reduce chronic cardiac allograft pathology, associating with LN structure remodeling [34]. Thus, there may be a place for oral administration of probiotics to facilitate graft survival.

The impact of extra-intestinal microbiota is also emerging. In humans, changes in lung microbiota have been associated with lung allograft outcomes, with restoration of the host microbiota in the transplanted lung correlating with better graft survival [25] and anabolic and catabolic remodeling of the transplanted lung being associated with distinct bacterial strains [35]. To address the impact of the graft microbiota on graft outcome, we used GF mice as hosts of sterile or mono-colonized skin grafts, where the gut was maintained sterile through the oral administration of non-absorbable antibiotics. Donor cutaneous colonization with *S. epi* was sufficient to accelerate skin graft rejection in the absence of intestinal colonization [36], demonstrating a definite causal role for the allograft's microbiota on transplant outcome. Surprisingly, skin *S. epi* did not increase alloreactive T cell priming in the skin graft-draining LNs, but instead might augment the effector immune response in the graft itself, as the skin graft but not the LNs expressed higher levels of inflammatory cytokine genes. This points to targeting the allograft's microbiota prior to transplantation as a potential strategy to improve graft survival. Together, these data suggest that both intestinal and graft microbiota may modulate alloreactivity and graft outcome, albeit by different mechanisms.

The microbiota and immunosuppressive drugs

While treatment with antibiotics is clearly expected to affect microbiota composition, a recent study that screened over 1,000 drugs with mammalian gene product targets reported that 24% of the tested drugs inhibited the growth of at least one bacterial strain [37]. Immunosuppressive drugs themselves have been found to drive some changes in microbial community structures [38,39], while cyclosporine A was reported to prevent post-transplant dysbiosis in rats transplanted with allogeneic livers [40]. Conversely, inter-personal microbiome variability has been associated with inter-personal differences in drug metabolism [41], affecting drug dosage. As proof of principle outside transplantation, *Eggerthella lenta* strains containing the gene for the reductase Cgr2 were found to inactivate the cardiac medication digoxin [42], such that individuals harboring such strains would need higher drug doses for equal activity. Whether specific bacterial strains metabolize immunosuppressive drugs is not known but fecal abundance of *Faecalibacterium prausnitzii* in renal transplant patients was associated with needing more tacrolimus within a month post-transplantation [43]. Whether *F. prausnitzii* itself metabolizes tacrolimus or whether its presence is a marker for other metabolizing communities remains to be determined, but the data suggest that inter- and intra-patient dosing variability could be ascribed to cross-sectional or longitudinal differences in microbiota composition, respectively.

The microbiota may also cause drug side effects. This has been well demonstrated with irinotecan, an anti-cancer chemotherapy drug. This drug is degraded systemically into

inactive metabolites excreted in the gut. People harboring intestinal bacteria expressing β -glucuronidases can reactivate these metabolites that then cause severe toxic diarrhea [44]. Drugs that inhibit bacterial P-glucuronidase prevent gut reactivation of irinotecan metabolites and diarrhea in mice, demonstrating beneficial effects of targeting biochemical pathways in commensal rather than mammalian cells [44]. Mycophenolate mofetil (MMF) is an immunosuppressant commonly prescribed to transplant patients that can also cause diarrhea. In mice, antibiotics prevented and reversed MMF's gastrointestinal toxicity and a GF status prevented it, suggesting its dependence on the microbiota [39]. Whether other transplant drug side effects are also microbiota-dependent, and by what mechanisms, remains to be studied.

The microbiota as a biomarker of transplant outcome or responsiveness to therapy

Another active area of investigation is whether the microbiota might be used as biomarker of transplant outcome. In a cross-sectional study, the ratio of firmicutes:proteobacteria was found to be lower in intestinal transplant recipients undergoing acute rejection than in non-rejectors [24] and changes in urinary microbiota correlated with signs of chronic rejection in kidney transplant recipients [45]. However, inter-personal variations in microbiota composition from either rectal, oral or urinary samples are so wide [23] that analysis of longitudinal changes in each patient might better predict danger to the allograft or susceptibility to immunosuppression weaning.

As mentioned above, assessing the relative abundance of *F. prausnitzii* might also help choose the appropriate dose of tacrolimus [43]. Similarly, in the cancer field, the fecal microbiota composition in metastatic cancer patients prior to initiation of checkpoint blockade immunotherapy was recently shown to be predictive of subsequent responsiveness to therapy [46–48]. Importantly, anti-tumor immunity and susceptibility to anti-PDL1 therapy was transferable into melanoma-bearing GF mice by FMT from responder versus non-responder patient samples [46], showing not only sufficiency of microbiota from responders to transfer this phenotype, but also the conserved immune impact from human to mouse. Similar experiments could be performed in transplantation.

The microbiota as immune-modulating therapy

If shifts in microbiota can cause immune phenotypes, it makes sense to develop strategies to control microbial community structures through dietary interventions, antibiotics or probiotics. For instance, gut bacteria promoted by high fiber diet alleviated type 2 diabetes [49]. In experimental transplantation, diets known to alter the microbiota such as high salt and high fat diet accelerated graft rejection [50–52] and hyperlipidemia prevented tolerance induction [53]. As another lifestyle intervention, exercise, which is also known to alter the microbiota [54], prolonged skin graft survival in mice [55]. Causality of the microbial changes induced by diet or exercise on allograft outcome has not been established.

Although use of antibiotics is probably too crude at the moment to reliably shape the microbiota and also risks promoting antibiotic-resistant bacterial strains, there may be a

place for narrow spectrum antibiotics if specific bacterial strains are proven to be dominantly pro-rejection. Bacterial strain-specific-targeting viral phages can also be developed as a pointed intervention, and commercial phages that target *Listeria* and *Salmonella* are already FDA approved. As mentioned above, strategies that target bacterial enzymes can also be considered, as are being tested to eliminate the intestinal toxicity of irinotecan [44].

FMT is now a well-accepted strategy to treat *Clostridium difficile*-driven diarrhea [56], and third party FMT appears safe following hematopoietic stem cell transplantation (HSCT) to maintain intestinal microbial diversity [57], the loss of which is associated with bacterial domination and sepsis [58]. However, selection criteria for donors of FMT need to be refined as transfer of pathogens asymptotically associated with the donor can be life-threatening in the recipient. In solid organ transplantation, one could consider autologous FMT from pre-transplantation banking, as currently tested in clinical trials post-HSCT ([ClinicalTrials.gov NCT03061097](https://clinicaltrials.gov/ct2/show/study/NCT03061097)). Alternatively, administration of clinical grade probiotics is attractive, but engraftment in recipients can be variable [59]. Interestingly, it might be possible to engineer probiotics to engraft better, as shown in a recent report in which transfer of a cluster of genes allowing utilization of porphyran, a marine polysaccharide not utilized by most bacterial strains, in conjunction to porphyran dietary supplementation, enabled titrated control of probiotic engraftment across hosts [60]. *B. pseudolongum* is the only commensal so far whose administration was sufficient to improve transplant outcome experimentally [34]. Whether this or other strains have similar effects in humans is important to pursue. In the cancer field, other strains of *Bifidobacterium* improved anti-tumor immunity and responsiveness to checkpoint blockade therapy in mice [61] and are already in clinical trials in metastatic cancer patients ([ClinicalTrials.gov NCT03595683](https://clinicaltrials.gov/ct2/show/study/NCT03595683) and [NCT03775850](https://clinicaltrials.gov/ct2/show/study/NCT03775850)), supporting the potential for translation.

Conclusion

The impact of the microbiota in transplantation is only starting to be appreciated. Much more work needs to be done to identify the exact mechanisms by which the microbiota modulates alloimmunity, the specific microbial strains or community structures that participate in this process, the interaction between the microbiota and immunosuppression, and what constitutes a healthy microbiota for transplant patients. Additionally, most work has focused on the role of bacteria. Studying the effects of resident fungi and viruses that are also part of the microbiota is an entirely open field, and changes post-transplantation are starting to be described in patients [62–65].

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Key Points

- Inter-individual variability in microbiota composition results in different transplant outcomes and explains in part experimental variability
- Some microbiota community structures accelerate, whereas others delay and yet others are neutral to transplant survival
- Both the intestinal microbiota and the microbiota at colonized organs modulate alloreactivity and graft outcome, albeit at different phases of the alloimmune response
- The fecal abundance of a specific commensal is associated with inter-personal variability in tacrolimus dose requirement
- The microbiota is responsible for the gastrointestinal toxicity of MMF

Figure 1. Potential intersections between the microbiota and transplantation.

The microbiota may be modified by the pre-transplant disease, the surgical injury, and the post-transplantation therapies, resulting in dysbiosis. The gut microbiota may metabolize immunosuppressive drugs causing inter-personal dosing variability or enabling drug side effects. The intestinal microbiota modulates the alloimmune response distally to the gut, at the priming phase, thus affecting outcome of both colonized and sterile grafts. The microbiota at colonized allografts may also tune the local alloimmune response, at the effector phase, further modifying outcome of colonized transplants. The gut microbiota may also become a useful biomarker to predict graft outcome, responsiveness to therapy, or to identify patients susceptible to specific drug side effects. Finally the microbiota may be modified for therapeutic purposes by diet, exercise, probiotics, anti-microbials, or drugs that target bacterial pathways.