

# Involvement of Gut Microbiota in Solid Organ Transplantation

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## ABSTRACT

Organ transplantation is considered the main therapeutic approach for the treatment of a wide variety of end-stage diseases. Transplantation success rate is dependent on the type of engrafted organs, as well as on the different kinetics of inflammation and immune-mediated responses towards donor antigens during the process. Several environmental factors seem to influence solid organ transplantation (SOT) outcomes, especially the composition of the donor's gut microbiota. Gut microbiota acts as a critical player in the process of maturation and modeling of immune responses, modulating not only local but also systemic immune responses. Emerging evidence from animal and human studies have shown that end-stage disease followed by SOT (e.g. kidney, small bowel, liver, lung, and heart transplantation) can significantly change gut microbial populations. These changes result in a wide range of outcomes, including intense alloimmune responses, characterized by high frequency of Th1 and Th17 CD4<sup>+</sup> T cells. Even though there has been significant progress in the field, it is still important to better characterize the changes in the gut microbiota populations and the mechanisms by which the host immune responses are influenced, which could contribute to additional intervention strategies aimed at improving graft and patient survival. Therefore, this review explores the positive and the negative effects of the gut microbiota in SOT.

## ABBREVIATIONS

AAMR: Acute antibody-mediated rejection; ACR: Acute cellular rejection; APC: Antigen-presenting cell; CGR: Chronic graft rejection; CKD: Chronic kidney disease; CTL: Cytotoxic T cell; DC: Dendritic cell; HLA: Human leukocyte antigen; LPS: Lipopolysaccharide; LT: Liver transplantation; LuT: Lung transplantation; MHC: Major histocompatibility complex; MMF: Mycophenolate mofetil; MVI: Microvascular inflammation; NF- $\kappa$ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; NK cell: Natural killer cell; PCR: Polymerase chain reaction; SBT: Small bowel transplantation; SCFAs: Short-chain fatty acids; SOT: Solid organ transplantation; TMAO: Trimethylamine-N-oxide; TNF- $\alpha$ : Tumor necrosis factor alpha.

## INTRODUCTION

The impact of the gut microbiota on solid organ transplantation (SOT) has been recently accepted and it is closely linked to graft function, once the microbiota could independently influence the host metabolic homeostasis<sup>1</sup>. The set of microorganisms that colonizes the several tissues of the human body, collectively termed the 'microbiota', is already well known for playing a key role in maintaining a state of immune homeostasis<sup>2</sup>. However, the influence of gut microbiota diversity and composition shifts on patient and graft survivals needs to be elucidated. It is not yet evident whether changes in bacterial composition are cause or an effect of distinctive graft outcomes<sup>3-5</sup>.

Although organ transplantation has become a valuable therapy for a range of end-stage diseases, the post-transplant complications (e.g. infections



and alloimmune rejections) over the years show that the therapeutic effectiveness of SOT is significantly reduced and needs further improvement. It is already established that the kinetics of graft rejection depends mainly on the extent of genetic differences between recipient and donor antigens, since allelic HLA discrepancies can result in distinct epitopes identified by alloreactive host T cells. Nonetheless, it is still unclear whether environmental factors, such as gut microbiota, could directly modulate the severity of the alloresponse<sup>5,6</sup>. In the context of SOT, the value and impact of the gut microbiota can be extremely variable and organ-specific. Organs considered sterile without local microbiota, such as kidney and heart, have better graft outcomes than organs colonized with microorganisms, such as lung, skin and intestine<sup>5,7</sup>. Partly, this disparity can be explained by the fact that these ‘non-sterile grafts’ may be affected by both the local microbiome (local effect on organ functionality) and the gut microbiota (systemic immune response on allograft). On the other hand, ‘sterile grafts’ may only be impacted by the adaptive immune response “taught” by the gut microbiota<sup>5,7</sup>.

#### THE IMMUNE SYSTEM IN THE CONTEXT OF SOT OUTCOMES

It is well described in the literature the involvement of the gut microbiota in the shape of the host immune system. However, little is known about how the gut microbiota is able to modulate the milieu and the immune reactions of organs that have their own set of microorganisms<sup>8</sup>. An example of this association between gut microbiota and immune system was demonstrated by Muri et al<sup>9</sup>, who observed that children with type 1 diabetes had an altered *Firmicutes/Bacteroidetes* ratio when compared to healthy children<sup>10</sup>. Similar observations, but in a mice model of transplant, concluded that commensal microorganisms, such as *Listeria monocytogenes* or *Staphylococcus aureus* could negatively modulate the alloreactivity in the context of sterile SOT or in a skin transplant<sup>11,12</sup>.

Due to the wide variety of transplanted organs, cases of rejection can be presented in three different scenarios (hyperacute, acute, and chronic rejections), according to the time of rejection and the immune effector mechanisms involved<sup>13</sup>.

First, hyperacute rejection usually happens in a short time after the transplant, generally minutes or hours after reperfusion and it is considered the

most severe form of rejection, accompanied by graft thrombosis and necrosis. Immunologically, these events are triggered by the presence of natural host antibodies against HLA antigens or carbohydrate antigens expressed by donor endothelial cells<sup>14</sup>. These natural antibodies are able to fix complement soluble proteins, resulting in endothelial cells damage and exposing their basement membrane and stimulating the secretion of von Willebrand factor, as well as uncovering the sub-endothelial Tissue Factor (CD142)<sup>15</sup>. Furthermore, the activation of humoral elements increases platelet adhesion and aggregation, reduces the blood flow in the graft and, consequently, causes graft loss<sup>16</sup>. The existence of natural antibodies (IgM) is believed to be the result of B1 cell stimulation by carbohydrates expressed by gut microbiota, such as levan (generated by enteric bacteria) and peptidoglycan polysaccharide complex (obtained from anaerobic bacteria) shown to be structurally similar to A/B blood antigens<sup>17,18</sup>. Fortunately, the search for anti-donor antibodies through cross-matching has reduced the frequency of hyperacute rejection and increased SOT success rates<sup>19</sup>.

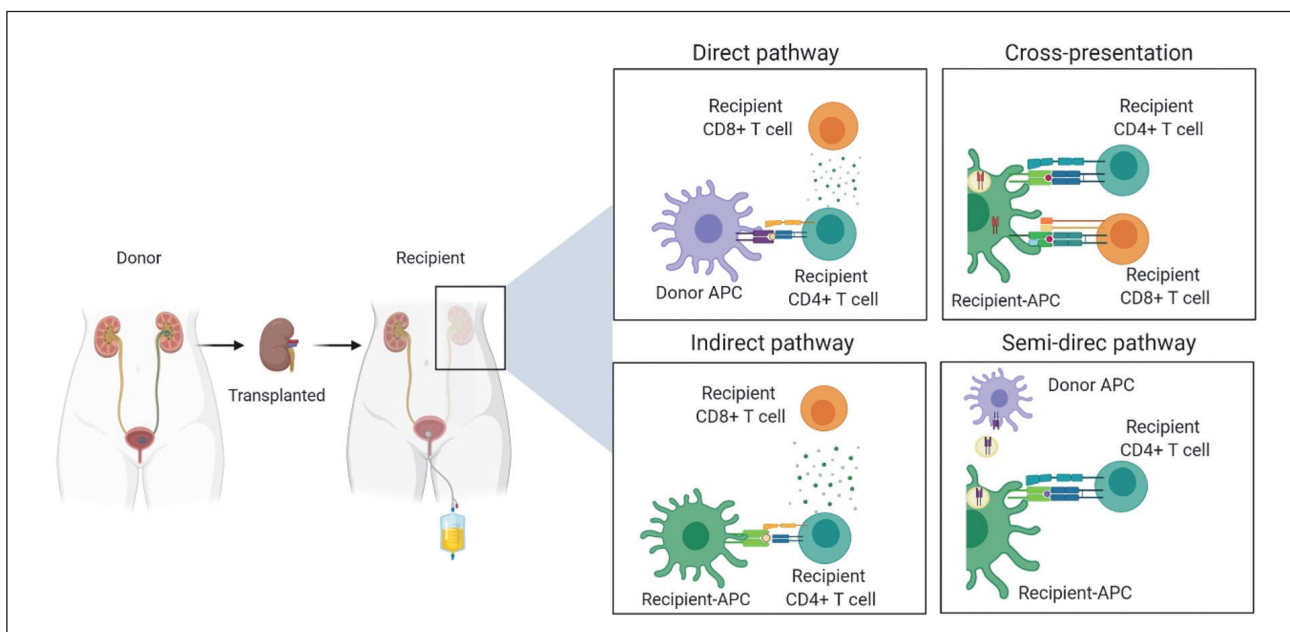
In contrast to the hyperacute form, the acute cellular rejection (ACR) appears days or months after transplantation, and shares some pathophysiological similarities with the hyperacute process, such as endothelial lesion, formation of fibrin thrombi, massive presence of polymorphonuclear leukocytes and graft necrosis<sup>20,21</sup>. The existence of anti-HLA antibodies and the deposition of the C4d complement fraction in capillaries classify the condition as acute antibody-mediated rejection (AAMR)<sup>22-24</sup>. Despite this, the main rejection mechanism is developed through the direct recognition of donor major histocompatibility complex (MHC) molecules by T cells, which induces an adaptive immune response with the generation of alloreactive T cells<sup>13, 25</sup>.

Similar to hyperacute rejection reactions, several studies have demonstrated the importance of the microbiota in the generation of ACR. Human and animal transplantation studies have established that CD4<sup>+</sup> T cells play an important duty in the generation of tissue/immunological damage associated with ACR<sup>26-28</sup>. An example of this interaction is observed in germ-free mice or antibiotic-treated mice, which have ‘disturbed’ immune system. In these models, it was observed an absence of Th17 cells, as well as lower Th1/Th2 cell ratio and morphological changes in the T cell zone in the lymph nodes<sup>2,29</sup>. Based on

these premises, by using antibiotics pretreated mice, Lei et al<sup>5</sup> observed a decrease in the frequency of CD4<sup>+</sup> T cells, which was able to minimize alloreactivity generated after tail skin transplant. McIntosh et al<sup>30</sup> developed another example of how the microbiota interferes with the transplant outcomes. In this study, two different groups of animals, with the same genetic background and variation in the constitution of the intestinal microbiota due to distinct animal service sources, showed different outcomes after skin transplantation. After closer evaluation, they found out that the presence of bacteria from the genus *Alistipes*, which produces sulfobacin B and was associated with better outcomes. Sulfobacin B<sup>31</sup> is an anti-inflammatory product able to interfere with tumor necrosis factor alpha (TNF- $\alpha$ ) production and translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) into the nucleus that could have a positive effect on the transplant outcomes<sup>32</sup>.

As previously mentioned, the first stage necessary for the assembly of the immune response involved in ACR is the presentation of MHC peptides by the antigen-presenting cell (APC). This can hap-

pen through three routes: direct, indirect and, less investigated, semidirect pathway (Figure 1). In the direct pathway, allogenic dendritic cell (DC), with incompatible MHC, directly primes recipient CD4<sup>+</sup> T cell, without the need for antigen processing<sup>33-35</sup>. On the other hand, the indirect pathway relies on the processing and presentation of donor-antigens by recipient APCs to MHC II-restricted CD4<sup>+</sup> T cells, which can secrete cytokine and signal to cytotoxic T cell (CTL)<sup>36</sup>. In the last route of recognition, the semi-direct route, the recipient APC acquires the intact donor MHC complex through cross-dressing<sup>37</sup>. More recently, several studies have shown that this MHC complex exchange can happen through trogocytosis<sup>38,39</sup>, which is a type of cell communication based on share of some plasmatic membrane portions, as well as some membrane-associated proteins<sup>40</sup>. Indeed, the transfer of these MHC complexes can promote the activation of CD4<sup>+</sup> T cells, which help CD8<sup>+</sup> T cells via co-stimulatory signals. This mechanism is characterized by the formation of a three-cell cluster, in which the same DC presents epitopes to a CD4<sup>+</sup> and a CD8<sup>+</sup> T cell<sup>38, 41</sup>.



**Figure 1.** Pathways of allorecognition. After kidney transplantation, graft rejection episodes can be initiated by different mechanisms. The direct route involves the direct presentation of donor MHC peptides by donor-APC to recipient CD4<sup>+</sup> T cell. In the indirect route, the recipient APC uptakes and processes donor antigens, followed by recipient CD4<sup>+</sup> T cell recognition, which can help CD8<sup>+</sup> T cell. In the semidirect pathway, there is a transfer of intact MHC molecules to the recipient APC, which presents to the CD4<sup>+</sup> T cell. Another mechanism able to induce allorecognition occurs through the cross-presentation by MHC-I and CD8<sup>+</sup> T cell. This Figure was created with BioRender. Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex.

After recognition through these three non-excluded routes, auxiliary T cell can be activated and differentiated into distinct effector subsets depending on microenvironment signals, such as Th1, Th2, Th17 and Treg<sup>42</sup>. The disbalance in the proportion of these subtypes or the moment when the molecules produced by them are secreted are known to be involved in some cases of ACR or tolerance transplantation. For instance, Th1 CD4<sup>+</sup> T cells are well known for being involved in the ACR, through the maintenance of proinflammatory milieu with high levels of IFN- $\gamma$  and IL-12. Like CD4<sup>+</sup> Th1 cells, Th17 cells may also be involved in ACR events, due to the high concentrations of IL-17 found in kidney biopsy samples of ACR patients<sup>43</sup>. In addition, Chung et al<sup>44</sup> showed an inverse relationship between FOXP3/IL-17 ratio and the worst prognosis of allograft dysfunction.

Besides CD4<sup>+</sup> T cells, direct recognition of alloreactive CD8<sup>+</sup> T cells is other mechanism involved in ACR<sup>41,45,46</sup>. The intact MHC-I complex is able to activate cytotoxic effector mechanisms that will result in the graft damage<sup>38,39</sup>. Often, the activation process of CTL CD8<sup>+</sup> T cells needs the help of other immune cells, such as APC and CD4<sup>+</sup> T cells<sup>47,48</sup>. However, Gelman et al<sup>49</sup> showed that CD4<sup>+</sup> T cells are not essential in ACR, suggesting that the allorecognition and cytotoxicity of CD8<sup>+</sup> T cells could occur directly through the presentation by donor-endothelial cells<sup>50</sup>.

Finally, chronic graft rejection (CGR) is considered the main cause of long-term graft loss. In contrast to the hyperacute and acute rejections, chronic rejection usually happens years after transplantation, and is caused mostly by sustained low grade adaptive immune/T cell responses<sup>13</sup>. Pathophysiological evidence showed a large infiltration of T cells in parenchyma grafts, which culminates in microvascular inflammation (MVI)<sup>20</sup>, causing vascular occlusion and hyperplasia<sup>13,51</sup>. These findings were accompanied by the presence of antibody and C4d deposition in graft capillaries<sup>52,53</sup>. In addition, recent work has shown that endothelial cells from donor are incapable of producing inhibitory signals to host NK cells (on account of the MHC mismatch), which provokes endothelial damage<sup>54</sup>. Moreover, a group of terminally differentiated effector memory CD8<sup>+</sup> T cells (TEMRA)<sup>55,56</sup>, which expresses large amounts of granzyme-B and perforin, was related to high risk of developing chronic dysfunction un-

til the 15th year after transplantation<sup>55</sup>. Up to now, it has been shown that the immune system plays an important role in the rejection of solid organs, either through the generation of alloreactive T cells or the generation of alloantibodies (Figure 1).

Moreover, various studies have described that the gut microbiota acts as a critical player in the process of maturation and modeling of adaptive immune responses. Certain commensal microorganisms induce B cell activation and generation of natural antibodies, which may mediate the processes of hyperacute and acute rejections. Likewise, these commensals directly influence the generation of APC and the activation of T cells. Nevertheless, whether the relationship between these microorganisms and SOT outcomes is positive or negative will be discussed in more detail in this review.

#### **REGULATORY MECHANISMS OF THE LOCAL AND SYSTEMIC IMMUNE RESPONSES MEDIATED BY GUT MICROBIOTA**

Gut microbiota is engaged in the regulation of gene expression, nutrient availability, body homeostasis and immune system development<sup>57</sup>. The microbiota represents a huge assemblage of “non-self” components and has a role in the interaction between local and systemic immune systems. Indeed, the microbiota “teaches” the immune system how to distinguish between risky life-threatening “non-self” components to induce immunity, and harmless beneficial “non-self” components to induce tolerance<sup>6,58</sup>. This “learning” process influences the onset and development of diseases, including intestinal and non-intestinal pathologies, such as obesity, diabetes mellitus and atherosclerosis<sup>57,59,60</sup>.

Generally, intestinal dysbiosis is correlated with shifts in intestinal immune system with increased permeability, inflammation, and compromised tolerance to food/microbial antigens. This increase of intestine barrier permeability contributes to a dysregulated activation of immune system and prolonged production of pro-inflammatory cytokines (“cytokine storm”) that leads to systemic inflammation<sup>61,62</sup>. Furthermore, intestinal dysbiosis could lead to a deregulation of innate immunity that is able to exacerbate graft inflammation and trigger alloreactive T cells (microbial antigen specific memory T cells). This may occur due to cross-reactivity with donor MHC antigens, which ultimately results in the induction of graft rejection<sup>62,63</sup>.

## GUT MICROBIOTA:

### CONSIDERATIONS FOR ALLOTRANSPLANTATION

The microbiota can be significantly altered by end-stage organ diseases and their consequent allotransplantation, therefore, it is crucial to find out the precise elements of the gut microbiota that could be protective or detrimental for the patient and/or graft survival<sup>64,65</sup>. Unfortunately, it is challenging to establish causality in humans. Therefore, animal experiments (e.g. germ-free and antibiotic-treated mice) are used to study whether the intestinal dysbiosis is a consequence or a player in the modulation of alloimmunity and graft outcomes<sup>66</sup>. Emerging evidence from human and animal research has revealed that microbial populations from intestine are modified in allogeneic transplant patients, with the potential to influence patient health and adversely affect allograft outcomes<sup>6,7,64,67</sup>.

A growing number of studies reporting disturbances in microbiota populations in SOT (kidneys, small bowel, liver, lung, and heart) suggests an association between intestinal dysbiosis with a broad range of outcomes, including distinct alloimmune responses to transplanted organs. In uremic rats and end-stage renal patients, Vaziri et al<sup>68</sup> showed a large disparity in 190 bacterial operational taxonomic units with remarkable raises in the phyla Firmicutes, Actinobacteria, and Proteobacteria. These observations can be partially explained by the change of intestinal luminal environment, especially because of the inflow in the luminal gut of uric acid, oxalate and urea, with a consequent disruption of the colonic epithelial tight junctions, which contributes to systemic inflammation<sup>68,69</sup>. In mice, Yang et al<sup>70</sup> showed that the chronic kidney disease (CKD) group exhibited a reduced bacterial biodiversity, with decline in *Lactobacillus* species and growth of *Clostridium* species, indicating an association between dysbiosis, amplified intestinal permeability, deviant mucosal immunity, systemic inflammation and fibrosis exacerbation in CKD<sup>70</sup>. Analysis of fecal bacterial composition in human patients showed that the abundance of Proteobacteria phyla was significantly higher in post-kidney transplantation samples than pre-transplant samples<sup>71</sup>. Also, differences in gut microbiota composition were observed among patients with post-transplantation diarrhea, with expressively lesser abundance of *Coprococcus*, *Bacteroides*, *Dorea*, and *Ruminococcus* genera. On the other hand, patients with acute kidney rejection exhibit-

ed a lower frequency of Bacteroidetes phylum and, at the order level, a significantly higher abundance of *Lactobacillales*, *Enterococcus*, *Anaerofilum* and *Clostridium*, suggesting that persistent variation in microbiota was an outcome of the transplant event and associated drugs<sup>71,72</sup>. Also, in mice, Wu et al<sup>73</sup> demonstrated a significant loss of gut microbiota diversity after kidney transplantation, driven predominantly by host-donor immune response, with significantly increased of Verrucomicrobia phyla, wholly because of genus *Akkermansia muciniphila*.

Similar to kidney allografts, the small-bowel allografts were linked to microbiota alterations, which exacerbated allograft rejection about 190 days after small bowel transplantation (SBT)<sup>74</sup>. Analysis of gut microbiome revealed that gut microbiota composition in rats experiencing chronic rejection was shifted towards greater bacterial abundance of *Escherichia coli*, *Clostridium spp.* and *Bacteroides spp.*, while Lactobacillales bacterial abundance was intensively reduced in the intestine<sup>74</sup>. In another human study, Oh et al<sup>75</sup> reported a connection of dysbiosis with a rise in the phylum Proteobacteria (family *Enterobacteriaceae*, especially genera *Escherichia* and *Klebsiella*) and a decrease in sundry members of the Firmicutes phylum (order Lactobacillales) in transplant patients experiencing rejection. This observation suggests that this microbial profiling may be a specific indicator of rejection, with a potential possibility to be a diagnostic indicator of SBT rejection that could be used in combination with current diagnostic tools to monitor SBT.

Similarly, liver transplantation (LT) is correlated with significantly modification of gut microbiota. In a study with 190 participants, Wu et al<sup>76</sup> observed that *Enterobacteriaceae* and *Enterococcus spp.* were significantly increased in LT patients, while *Faecalibacterium prausnitzii*, *Bifidobacterium spp.* and *Lactobacillus spp.* were significantly lower compared to the control group<sup>76</sup>. Interestingly, Sun et al<sup>77</sup> observed gut microbiota differences between patients awaiting LT and healthy controls. In the same work, differences were found in the gut microbiome of pre-LT and post-LT patients, with a significant decrease in *Actinobacillus*, *Shigella* and *Escherichia* abundance, and an increase in *Micromonosporaceae*, *Eubacteriaceae* (especially *Sarcina* genus), *Desulfobacterales*, and *Akkermansia* abundance<sup>77</sup>. Moreover, a longitudinal study of gut microbiota in LT reported, at family level,

a decrease in *Enterococcaceae*, *Peptostreptococcaceae*, *Lactobacillaceae*, *Ruminococcaceae*, and *Clostridiaceae*, whereas an increase of *Bifidobacteriaceae*, *Enterobacteriaceae*, *Bacteroides* and *Streptococcaceae* was seen in patients with ACR<sup>78</sup>. In a rat LT model, Ren et al<sup>79</sup> found that the phylum Firmicutes (especially *Faecalibacterium prausnitzii* and *Lactobacillus*) was decreased during ACR, while the abundance of Bacteroidetes phylum was substantially increased.

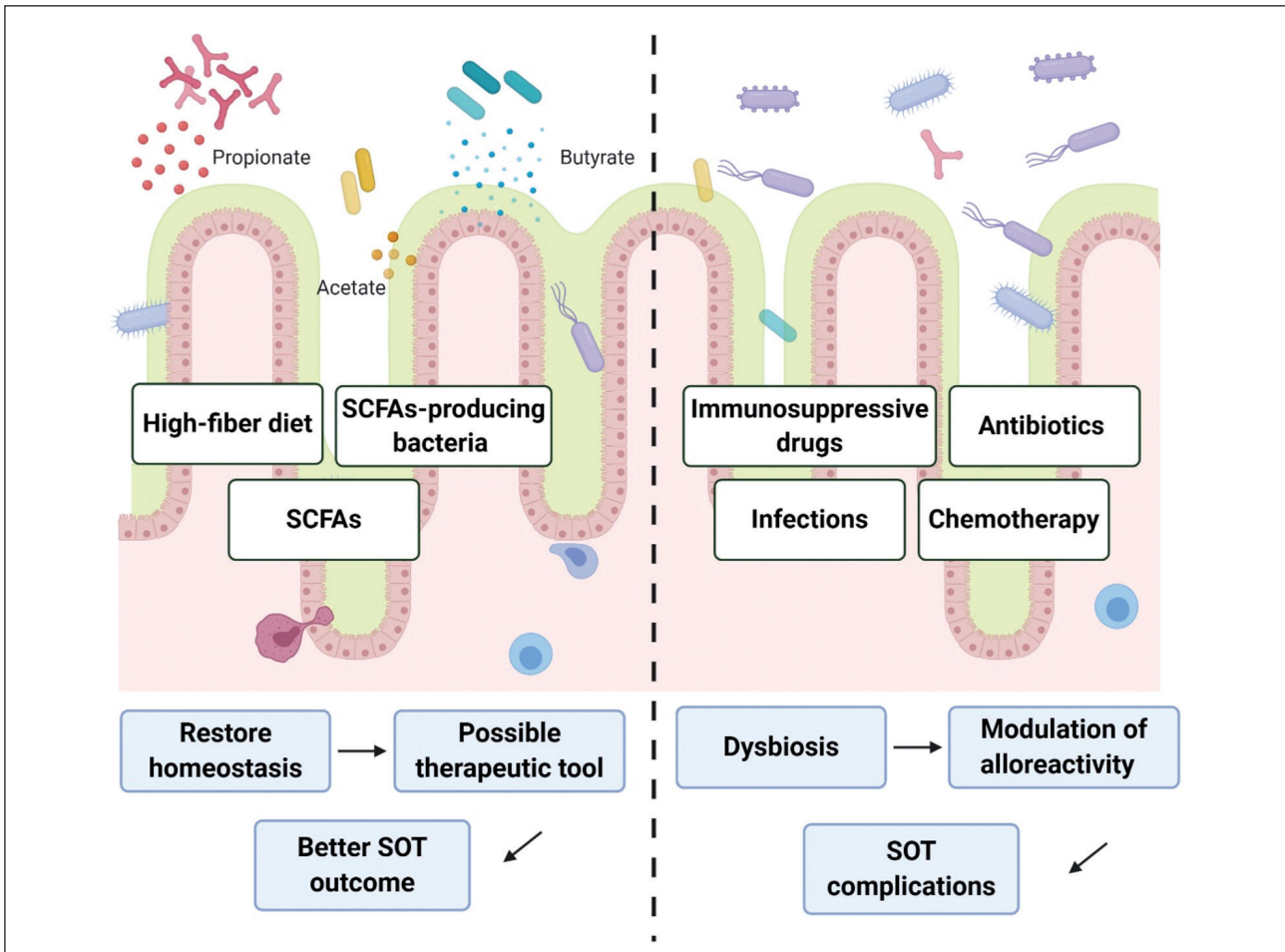
Different from other SOT, no human studies were found that characterized the changes in the gut microbiome in lung transplantation (LuT). One study reported an association of gut microbiota depletion with a reduction in the severity of rejection 21 days after LuT in broad-spectrum antibiotic pretreated mice<sup>80</sup>. Nonetheless, several studies reported local microbiota changes in LuT. For instance, Charlson et al<sup>81</sup> showed a reduction in bacterial and fungal diversity in LuT recipients compared to non-transplant controls. Also, Bernasconi et al<sup>82</sup> associated dysbiosis in the lung with inflammatory and remodeling profiles, linking neutrophilic and macrophage infiltration and histological inflammation with Firmicutes or Proteobacteria dysbiosis. Furthermore, other studies suggested a probable causal connection between local bacteria and rejection, as observed in LuT patients, who exhibited an association between the beginning of bronchiolitis obliterans syndrome (one of the main causes of the loss of lung transplant) and alterations in the pre-transplant microbiota<sup>83</sup>. Interestingly, positive PCR for *Simkania negevensis* in bronchoalveolar lavage preceded acute rejection in 37% of LuT patients and its presence was associated to a 3.4-fold higher likelihood of developing acute rejection<sup>84</sup>. Nevertheless, further studies are still necessary to clarify the interplay between local bacterial changes and the gut microbiota in LuT.

In the setting of cardiac allograft, studies have not investigated a putative connection between heart transplantation and gut microbiota, even though metabolites of the gut microbiota, like trimethylamine-N-oxide (TMAO), have attracted enormous attention due to the potential role for increased cardiovascular risk<sup>67,85</sup>. Notably, studies with germ-free and antibiotics-treated mice demonstrated that TMAO generation was dependent on the gut microbiota<sup>85</sup> and higher plasma TMAO levels were correlated with the main risk of cardiac incidents. Therefore, TMAO levels may

represent an important predictive marker for long-term mortality in acute coronary syndromes<sup>86</sup>. Nevertheless, it is worth mentioning the difficulty in linking heart disease causality with gut microbiota and TMAO, because this relationship involves complex functional genomics and metabolic profiling<sup>85</sup>. Moreover, the microscopic species and pathways that participate not only in TMAO synthesis, but in the production of all gut metabolites, remain to be elucidated and studies focusing on the influence of gut microbiota and its metabolites in heart transplantation are still needed.

Overall, these numerous works have studied the alteration of gut microbiota after SOT and demonstrated that loss of microbial diversity following transplantation, including shifts toward dysbiosis, has been correlated with posttransplant diarrhea, longer hospital stays and more severe posttransplant infections<sup>64,67,71</sup>. Besides these complications, SOT patients are exposed to diverse treatments, such as antibiotics, preparative regimens prior to transplantation, chemotherapy and immunosuppressive drugs that can alter gut microbiota, resulting in intestinal imbalance or dysbiosis<sup>7,64</sup>. This dysbiosis can provide tonic inflammatory signals that can induce alloreactivity that could promote immune graft rejection<sup>30,62,65</sup> (Figure 2).

But on the other side, gut microbiota produces and releases metabolites, like short-chain fatty acids (SCFAs), tyrosine and tryptophan, which have an impact on distal immune response and could modulate allograft outcomes<sup>67,87</sup>. One of the major products of intestinal microbial metabolism are SCFAs (mainly acetic, propionic, and butyric acid), which are generated from fermentation of nondigestible polysaccharides and play a critical role in the maintenance of gut and immune homeostasis<sup>87,88</sup>. Interestingly, in a hypertensive mice model, Marques et al<sup>89</sup> showed that elevated ingestion of fiber modified the gut microbiota populations and raised the abundance of acetate-producing bacteria, decreasing the systolic and diastolic blood pressure, and cardiac fibrosis. Also, Wu et al<sup>73</sup> demonstrated that the consumption of a high-fiber diet in a murine kidney transplantation model leads to significant growth in species known to produce SCFAs, including *Bacteroides spp.* and *Bifidobacterium spp.*, with prevention of dysbiosis and reduction of allograft rejection compared to normal chow fed group. Moreover, supplementation with acetate demon-



**Figure 2.** The gut microbiota balance. Several studies demonstrated that diverse transplant-related factors, such as antibiotics, infections, chemotherapy and immunosuppressive drugs, may negatively affect gut microbiota composition, resulting in intestinal dysbiosis. Moreover, the gut microbiota is intimately involved in the modulation of alloreactivity locally and systemically, and dysbiosis seems to have a role in SOT complications. On the other hand, therapeutic dietary interventions using gut microbiota components and its metabolites (high fiber diets, SCFAs and SCFA-producing bacteria) could potentially compensate the intestinal imbalance, reestablishing a healthy microbial community in the intestine of SOT patients. Therefore, dietary supplementation with prebiotics, probiotics or synbiotics is a putative therapeutic tool against SOT-related complications and may lead to better SOT outcomes. Abbreviations: SCFAs, short-chain fatty acids; SOT, solid organ transplantation. This Figure was created with BioRender.

strated equivalent results to those obtained with high fiber diet, including the protection of kidney allograft<sup>73</sup>. Therefore, based on the massive use of antibiotics and high frequency of dysbiosis in transplant patients, someone could speculate that reestablishing appropriate SCFAs levels in the intestine through replacement therapy could hypothetically balance the reduction of SCFA-producing bacteria and reinstate a proper metabolic and functional equilibrium<sup>64</sup>. Eguchi et al<sup>90</sup> described that perioperative administration of *Bifidobacterium breve*, *Lactobacillus casei* and galactooligosaccharides markedly decreased infections (e.g.

urinary tract infections with *Enterococcus spp.*) after elective living-donor LT, suggesting that synbiotic therapy (simultaneous administration of prebiotics and probiotics) is a valid therapeutic tool against SOT-related complications (Figure 2).

Interestingly, immune tolerance towards SOT can be abolished by inflammatory stimuli, including *Staphylococcus aureus*<sup>12</sup> or *Listeria monocytogenes*<sup>91</sup> infections. It was demonstrated that even after the cardiac allografts tolerance had been established in mice, it could be broken by *L. monocytogenes* infection<sup>11</sup>. Also, Lei et al<sup>5</sup> reported that depletion of gut microbes shortly before transplan-

tation in mice was sufficient to prolong survival of MHC class II-mismatched heart allografts and antigen-mismatched skin graft (tail skin from donor was transplanted onto the recipients' flank). Furthermore, in this study, alloimmunity seemed to be qualitatively rather than quantitatively modulated by microbiota, since material from untreated mice feces, but not from antibiotics-treated mice feces, stimulated alloreactive T cell priming by APCs, promoting graft rejection<sup>5</sup>.

It is important to highlight the complexity of the microbiota components as inflammatory stimuli that could promote immune graft rejection, since the microbiota has also regulatory effects that can prevent allograft rejection. Alhabbab et al<sup>92</sup> demonstrated that gut microbiota can rise the expansion of transitional IL-10-producing B cells, which prevents TNF- $\alpha$  production by T cells, prolonging allogeneic skin graft survival. Nevertheless, it is coherent to suggest that inflammatory stimuli may change the stability from regulatory to effector immune responses, stimulating innate immune mechanisms (DCs, monocytes), which, in turn, can cause nonspecific inflammation via preexisting T-cell immunity<sup>93</sup>.

#### MICROBIOTA AND IMMUNOSUPPRESSION IN ORGAN TRANSPLANTATION

Lastly, most types of transplant patients receive relatively the same immunosuppressant drugs (e.g., tacrolimus and mycophenolate mofetil, MMF) and the lifelong immunosuppression prevents immune-mediated ACR of the donor organ, but, at the same time, the patients are more susceptible to drug toxicity, cancer and infections. An investigation of the association between tacrolimus dosing needs and gut microbiota in adult kidney transplant recipients showed that high doses modified the frequency and composition of the gut microbiota<sup>94</sup>. Moreover, patients who needed higher doses of tacrolimus during the first month of transplantation had an altered gut microbiota with elevated fecal abundance of *Faecalibacterium prausnitzii* in comparison to the ones that did not need high tacrolimus doses<sup>94</sup>.

In rats, Bhat et al<sup>95</sup> showed that tacrolimus and rapamycin/sirolimus (an immunosuppressant isolated from the bacteria *Streptomyces hygroscopicus*) treatment can cause a shift of gut microbiome towards a catabolic microbial profile, with decreased abundance of bacterial species such as

*Staphylococcus ssp.*, *Roseburia ssp.* and *Oscillospira ssp.*, and an increase of *Akkermansia muciniphila*. They correlated these microbiota shifts caused by both drugs with alterations seen in gut microbiota of diabetic and obese patients, indicating a possible contribution of the gut microbiome to the post-transplant diabetes observed in a considerable subset of SOT patients<sup>95</sup>.

Treatment with MMF, another immunosuppressant commonly prescribed after transplantation, was also linked with deviations in gut microbiota composition, with reduction of global diversity and increase of *Shigella/Escherichia* species. Also, MMF has been associated with enrichment of genes encoding for enzymes involved in lipopolysaccharides (LPS) biosynthesis and high levels of LPS in serum and feces. Furthermore, gastrointestinal toxicity associated with MMF was likely mediated through the changes of gut microbiota due to the fact that MMF did not cause gut inflammation in germ-free or antibiotic-treated mice<sup>96</sup>.

It is important to highlight that immunosuppressant drugs have stronger effects than the sole decrease of alloimmune response in SOT patients. Although these reports provide insights into the influence of gut microbiota on immunosuppressive therapy, the relationship between pharmacokinetics of immunosuppressant drugs and gut microbiota requires future investigation.

#### CONCLUSIONS

Recipients of SOT express heterogeneity in the incidence and period of rejection episodes. Gut microbiota is intimately engaged in the modulation of alloreactivity locally and systemically and seems to be involved in posttransplant complications and allograft outcomes. Further studies are necessary to characterize changes in gut microbiota populations, as well as the mechanisms by which these microbes can influence immune responses (e.g. Treg induction, microbial peptide secretion with 400 modulatory and/or anti-inflammatory activities), and related outcomes in SOT patients (Table 1). A more complete comprehension of the bidirectional relationship between SOT and the gut microbiota is required to identify potential risk factors, such as unpredictable medication metabolism, infection, and rejection, which could contribute to additional intervention strategies aimed at improving graft survival.



**Table 1.** Clinical trials involving changes in microbiota and solid organ transplantation registered in the National Library of Medicine (NLM) database.

Study title	Organs evaluated	Status	Eligible ages	Location	NCT number
Intestinal Microbiome Dynamics in Solid Organ and Stem Cell Transplant Recipients	Kidney, liver, allogenic hematopoietic stem cells, and mesenchymal stem cell transplantation	Enrolling	Child, adult, and old adult	Belarus	NCT04281797
Fecal microbiota transplantation of <i>C. difficile</i> infection in solid organ transplant recipients	Without specification of the criterion	Phase 2 (suspended due to COVID-19)	Adult (> 18 years old), old adult	United States	NCT03617445
Characterization of the Intestinal Microbiome Evolution After Kidney Transplant Donation or Receipt (COMET-DR)	Renal transplant	Recruiting	Adult (> 18 years old), old adult	United States	NCT03043339
Human Microbiota and Liver Transplant	Liver transplant	Recruiting	Adult (18 years to 68 years)	Spain	NCT02223468
Immunosuppressive Drugs and Gut Microbiome: Pharmacokinetic- and Microbiome Diversity Effects (MicrobioTac)	Renal transplant	Recruiting	Adult (> 18 years old), old adult	Norway	NCT04207177
Bile Acids and Incretins in Pancreas Kidney Transplant Patients (ITABI)	Pancreas-kidney transplantation	Completed	Adult (18 years to 55 years)	France	NCT02234349

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**CONFLICT OF INTEREST:**

The authors declare that they have no conflict of interest to disclose.

**CONTRIBUTIONS:**

I.K.M.W. and N.O.S.C conceptualized the manuscript. I.K.M.W., D.G.S. and T.S.B.H. wrote the manuscript. I.K.M.W, A.P.S. and N.O.S.C gave theoretical support and revised the manuscript.

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