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Bacterial Infections, Alloimmunity, and Transplantation Tolerance

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Abstract

Transplantation of solid organs across histocompatibility barriers in the absence of immunosuppression is invariably followed by acute allograft rejection. Although several immunosuppressive regimens have been developed to prevent allograft rejection, these global immunosuppressive agents effectively inhibit all T cells leaving the host vulnerable to infections. Thus a major goal in transplantation immunology is to induce donor-specific tolerance that results in the extended suppression of allograft-specific immune responses, while leaving the remainder of the immune system competent to fight infections and malignancies. Initial successes in identifying approaches that successfully induce transplantation tolerance in experimental models have led to a newer research focus of identifying potential barriers to the induction of such tolerance as well as events that may reverse established allograft tolerance. Both clinical and experimental studies have identified bacterial infections as a possible trigger of allograft rejection. Recently, experimental models of transplantation tolerance have identified that bacterial signals can promote acute allograft rejection either by preventing the induction of transplantation tolerance or by reversing tolerance after it has been stably established. This review summarizes experimental and clinical literature supporting the hypothesis that bacterial infections and innate immunity can qualitatively and quantitatively alter adaptive alloreactivity through effects on innate immune responses.

Introduction

It has long been observed that there is a significant difference in the clinical outcomes of allografts depending on the transplanted organ (1). We noted that some organs with lower success rates, such as skin, lung and intestines, contain a higher commensal load compared to other organs, such as heart, kidney, and liver. These observations led to a hypothesis by

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our group that concomitant exposure of the immune system to bacterial motifs and alloantigens synergistically contribute to increased immune responses to the alloantigen, and ultimately to graft injury and rejection.

Acute allograft rejection is considered to be largely a T cell-mediated event, with the contribution of B cells and antibodies becoming increasingly appreciated (2). Clarification of innate immune responses modulating adaptive immune responses, and the identification of the potent pro-inflammatory events initiated by bacterial infections provide a theoretical framework for the hypothesis that bacterial infections function as potent adjuvants to enhance alloreactivity and allograft rejection (1). Several components of the innate immune response that are elicited by bacterial infections, including the engagement of pattern recognition receptors and the production of pro-inflammatory cytokines, are predicted and have been shown to be important in allograft rejection (3,4). Recent data from experimental studies, and the clinic, suggest that bacterial infections stimulate innate immunity and either directly, or through bystander effects, can qualitatively and quantitatively alter the magnitude of the alloreactive immune response (Figure 1).

Pattern Recognition Receptors (PRRs)

The pioneering studies by Janeway and Medzhitov on the importance of innate immunity in the generation of adaptive immune responses identified an important role for Toll-like receptors (TLRs) (5,6). TLRs are transmembrane molecules that recognize a variety of microbial molecular patterns. There are two major subsets of TLRs as summarized in Table 1. The first subset comprises TLRs in endosomal compartments that recognize nucleic acids specific for viral recognition (TLR3, TLR7, and TLR8) or unmethylated CpG motifs common to both viruses and bacteria (TLR9). The second subset of TLRs is located at the plasma membrane and recognizes primarily bacterial motifs (TLR1, TLR2, TLR4-6, TLR4, TLR5, and TLR11) (7). TLR engagement by ligands leads to downstream signaling events, activation of transcription factors including nuclear factor-kappa B (NF- κ B) and activator protein 1 (AP-1), and ultimately to the production of pro-inflammatory mediators and antimicrobial compounds that are important for the immune defense against bacterial, viral, and fungal infections. These mediators, together with TLR-induced upregulation of major histocompatibility complex (MHC), costimulatory molecule expression, and consequently increased antigen presentation, form the basis for how TLRs shape innate and adaptive immune responses (8). While TLRs are predominantly expressed on antigen presenting cells (APCs), namely macrophages and dendritic cells (DCs), their expression on T and B cells has also been reported (9-11). Ligation of TLRs on these cells directly promotes their survival, activates downstream signaling events and adaptive immunity (12,13), raising the possibility that ligation of these receptors by bacterial antigens may have direct immunostimulatory effects on CD4⁺CD25⁻ conventional T cells (Tconv) and B cells *in vivo*.

There is emerging evidence that TLRs are also expressed on regulatory T cells (Tregs) and may play an important role in enhancing or suppressing their function (14). With the exception of TLR3, which is not expressed on Tregs, and TLR10, which is not expressed on Tconv, both CD4⁺ cells types express the majority of the other TLRs (14). Additionally, Tregs express higher levels of TLR2, TLR4, TLR5, TLR7/8, and TLR10 than Tconv (15). Engagement of cell surface TLRs has been mostly reported to result in enhanced Treg function or survival, perhaps to reduce inflammation and potential septic shock, although this increased suppression sometimes follows transient downregulation of FoxP3 and loss of suppressive function such as occurs following TLR2 ligation in mouse Tregs (14,16-19). Various TLR2 agonists were recently reported to reduce suppressor function directly on human Tregs (20). Engagement of intracellular TLRs has been shown to reduce Treg-

mediated suppression (21-23), thus promoting Tconv function and potential viral elimination. The overall effect of TLR signaling on Tregs appears to favor suppression, as Tregs deficient in the adaptor molecule myeloid differentiation primary response gene 88 (MyD88), that is necessary for signaling by all TLRs except TLR3 (which is not expressed in Tregs), have reduced suppressive capacity *in vitro* and *in vivo* (24).

Several studies in rodent transplantation models have implicated TLR signaling in allograft rejection. Using a mouse model for minor antigen-mismatched skin grafts, Goldstein *et al.* showed that TLR signaling via the adapter protein MyD88 is necessary in both donor and recipient cells for rejection (25). In fully allogeneic murine heart or skin transplants, MyD88- deficiency in both donor and recipient animals only modestly prolonged graft survival (3). The simultaneous deletion of MyD88 and Toll-interleukin-1 receptor domain-containing adapter protein inducing interferon beta (Trif) resulted in significantly delayed rejection compared to the deletion of MyD88 or Trif alone (26). These observations suggest that TLR signaling can enhance allograft responses under settings where alloreactivity is limited, but not under circumstances when alloreactivity is robust.

Additional support for the notion that TLR signals enhance alloreactivity comes from studies in animal models in which alloreactivity is modulated by costimulation blockade. In the highly immunogenic mouse model of allogeneic skin transplantation, the absence of both recipient and donor MyD88-dependent TLR signaling synergized with costimulation blockade to mediate long-term graft survival (27). Complementary studies demonstrated that the co-administration of TLR agonists, including lipopolysaccharide (LPS) and CpG, when delivered at the time of transplantation, antagonized the effects of co-stimulation blockade (27,28). Engagement of TLRs and the subsequent elaboration of pro-inflammatory cytokines, such as interleukin (IL)-6, may result in enhanced responses of alloreactive T cells, impaired suppression of Tregs, and their skewing into T-helper (Th) 17 and/or Th1 cells (9,29). Furthermore, TLR-mediated production of Type I interferons (IFN) have the potential to act directly on alloreactive effector T cells (30).

Clinical literature also supports a link between TLR stimulation and human allograft rejection. TLR4 polymorphisms (Asp²⁹⁹Gly and Thr³⁹⁹Ile), associated with endotoxin hyporesponsiveness, have been described in the human population (31). Recipients heterozygous for these polymorphisms showed a lower incidence of lung and renal allograft rejection (32,33), but were also associated with more severe and frequent infectious episodes (34). The later point was confirmed in a study reporting an association of TLR4 and the risk of invasive aspergilosis among recipients of hematopoietic-cell transplantation from unrelated donors (35). More recently, Eid *et al* reported an association between homozygous TLR2 Arg754Gln polymorphism, but not TLR4, and liver allograft failure in recipients with chronic hepatitis C virus infection (36). Thus, both experimental and clinical models support a role for TLRs in enhancing alloreactivity, in addition to their roles in protective immunity to pathogens.

There are several other classes of PRRs including: RIG-I-like receptors, Nod-like receptors (NLRs), and C-type lectin receptors (CLRs) (37) that may play a role in allograft rejection in addition to TLRs (Table 1). NOD2 has recently been shown to negatively regulate the activity and function of host DCs. This is exemplified by increased alloactivation and proliferation of donor T cells in NOD2^{-/-} allogeneic bone marrow transplant recipients and aggravation of graft versus host disease (GVHD) (38). In clinical allogeneic hematopoietic stem cell transplantation (HSCT), several studies show a connection between NOD2 small nucleotide polymorphisms (SNPs) and increased GVHD incidence and severity (39,40). However, this relationship has not been borne out in other studies (41,42) suggesting that

polymorphisms in NOD2 genes can influence allogeneic HSCT, but multiple clinical parameters can obscure the contribution of NOD2 (38).

Endogenous damage-associated molecular patterns (DAMPs), or alarmins, are molecules released by damaged, necrotic, and some inflammatory cells. Several excellent reviews describe their contribution to the induction of sterile, or noninfectious, inflammation (43-47). There are a multitude of sequestered intracellular DAMPs including high mobility group box 1 (HMGB1) (48), uric acid (43), galectins (44), S100 proteins (45), and heat shock proteins (44) that may play a role in allograft rejection (Table 1). Indeed, several investigators have observed the contribution of HMGB1 to cardiac allograft rejection by showing that HMGB1 expression is upregulated in cardiac allografts and that blockade of HMGB1's biological functions prolonged graft survival (48,49). Additionally, since DAMPs are released by damaged or necrotic cells, and ischemia/reperfusion injury is an inescapable aspect of all transplant surgeries as well as downstream of infections, it remains possible for DAMPs to contribute to the initial proinflammatory events that promote acute and chronic allograft rejection (43-47). The list of PRRs and their ligands continues to expand, and their roles in transplantation will likely be investigated as reagents to do so become available. A brief list of PRRs and their role in transplantation is summarized in Table 1.

Impact of Bacterial Infections on Alloreactivity and the Induction of Transplantation Tolerance

The investigation of individual PRRs allows for precise mechanistic insights into how each PRR functions and impacts alloreactivity. However, the situation in the clinic is likely to be more complex, with the immune system encountering multiple types of PRRs, as well as DAMPs, during the transplantation process. In the post-transplantation period, it is likely that multiple and unique combinations of TLRs and DAMPs will be encountered during different types of infections. We have investigated the effects of bacterial infections on alloreactivity, initially using the intracellular Gram-positive *Listeria monocytogenes* as a model bacterial infection (4).

Indeed, peri-operative *Listeria* infection prevents cardiac and skin allograft acceptance induced by anti-CD154 and donor-specific transfusion (DST) in mice (4). We demonstrated that *Listeria*-mediated rejection is not due to the generation of cross-reactive T cells but rather to the pro-inflammatory events that are elicited by the infection. These pro-inflammatory events are independent of signaling via MyD88, as is the ability of *Listeria* infection to prevent the induction of tolerance, but are dependent on the expression of the phagosome-lysing pore-former listeriolysin O (LLO), which destroys the phagosomal membrane and allows phagocytosed *Listeria* to escape into the cytoplasm (4). The invasion of the cytosol triggers early inflammatory responses and, ultimately, protective immunity that depends both on innate and adaptive immune responses, including the production of MCP-1 (CCL2) and Type I IFNs in a MyD88-independent, but NF- κ B-dependent, manner (50). Protective immunity to *Listeria* has been shown to be independent of Type I IFN α/β R signaling (50). However, we showed that expression of IFN α/β R is absolutely essential for the ability of *Listeria* to prevent tolerance induction and that the administration of IFN- β , in a dose-dependent manner, is sufficient to prevent anti-CD154/DST-mediated prolongation of skin allograft survival (4).

We extended these observations to demonstrate that infection with Gram-positive *Staphylococcus aureus* also prevents allograft acceptance induced by anti-CD154/DST treatment (unpublished observations). However, in contrast to *Listeria* infection, the effect of *Staphylococcus* infection is dependent on MyD88 and on the production of IL-6. Collectively, our results suggest that bacterial exposure at the time of transplantation can

antagonize both tolerogenic as well as immunosuppressive regimens by enhancing alloantigen-specific immune responses, independent from the generation of cross-reactive memory T cells. Remarkably, we observed that some types of bacterial infection (*e.g. Pseudomonas aeruginosa*) have no effect on the induction of graft acceptance, in contrast to others that profoundly enhanced alloreactivity and prevented graft acceptance.

These observations are consistent with investigations into the impact of viral infections on alloreactivity and the induction of transplantation tolerance. Early studies by Welsh *et al.* showed that acute infection with lymphocytic choriomeningitis virus (LCMV) and pichinde virus, but not murine cytomegalovirus and vaccinia virus, one day after skin transplantation, induces allograft rejection in mice treated with DST and anti-CD154 antibody (51). Cook *et al.* demonstrated that latent CMV infection results in pro-inflammatory events associated with IFN- α expression in the allograft that disrupts the development of allograft acceptance (52). These data suggest that, in addition to the effects of heterologous immunity as a result of cross-reactive T cell responses between virus and alloantigen, the pro-inflammatory responses elicited to control a viral infection may also have a bystander effect on an ongoing alloreactive response.

Recent observations by Perona-Wright *et al.* provide a mechanistic basis for the bystander effect of cytokines produced during infections affecting the alloreactive response (53). By assessing cytokine signaling, they demonstrated that IFN- γ and IL-4, produced during *Heligmosomoides polygyrus* infection, signal to the majority of lymphocytes throughout the reactive lymph node, whether antigen-specific or not, thereby modifying their T helper-type polarization. Those observations challenge the current paradigm that cytokine production in lymphoid tissues is tightly localized to conjugate cells and suggest a mechanism by which cytokine conditioning of bystander alloreactive T cells as well as antigen-presenting cells during bacterial or viral infections could confer resistance to tolerance induction.

Impact of Bacterial Infections on the Maintenance of Transplantation Tolerance

With successful induction of transplantation tolerance reported in a number of preclinical models (54-56) and in limited clinical scenarios (57,58), understanding the circumstances under which the tolerant state can be breached has become an important issue that has to be addressed. This information is necessary if transplantation tolerance is to be able to achieve rates of stable and long-lasting allograft survival that are superior to current outcomes with pharmacological immunosuppression. While most of the investigations into barriers to tolerance have focused on what prevents the induction of tolerance, it is possible that events that prevent tolerance induction may differ from those that can break tolerance after it has been stably established. Indeed, a number of factors have been shown to prevent the induction of tolerance, including breadth of the memory and naïve alloreactive T cell repertoire (59), presence of memory and homeostatically expanded T cells (60,61), as well as memory B cells (2). These events may have less relevance once tolerance has been established. Likewise, the stimulation of innate immunity, via TLR engagement (27,28) or infections (4), prevents the development of allograft tolerance. However, such signals have not been reported to reverse established tolerance. It is therefore reasonable to theorize that established tolerance may be more difficult to abrogate, particularly since tolerance in many experimental models is likely to involve changes in the alloreactive immune repertoire, including clonal deletion, anergy and dominant suppression, as well as changes within the allograft itself in the form of an anti-inflammatory or accommodated phenotype (62,63).

We recently reported that infection with *Listeria monocytogenes* successfully abrogates cardiac transplantation tolerance in mice after it has been stably induced by costimulation-

blockade-based therapy, resulting in T cell-dependent acute rejection (64). Increased numbers of graft-infiltrating macrophages and dendritic cells, as well as CD4⁺FoxP3⁻ and CD8⁺ T cells, was observed during the rejection of the accepted allograft (64). Increased frequencies of alloreactive, as well as Listeria-reactive IFN- γ -producing T cells, was demonstrated, together with the inability of Listeria infection to precipitate the rejection of a freshly-transplanted or established syngeneic allograft, further suggesting that the reversal of tolerance is mediated, at least in part, by alloreactive T cells (64). The requirements for overturning tolerance with Listeria infection are more stringent compared to the prevention of this state. A 15-fold higher infectious dose of Listeria is required for the reversal of tolerance compared to preventing the induction of tolerance. The reversal of tolerance by Listeria infection requires the presence of both CD4⁺ and CD8⁺ T cells, while the prevention of tolerance by Listeria infection is dependent on either CD4⁺ or CD8⁺ T cells (4,64). It is currently unclear why both CD4⁺ or CD8⁺ T cells are necessary for breaking established tolerance, but we speculate that *in situ* mechanisms in place for maintaining tolerance in the secondary lymphoid organs, as well as in the graft, may blunt the ability of effector T cells to mediate rejection, resulting in the necessity of both CD4⁺ and CD8⁺ effector T cells. Alternatively, the absence of alloantibody production when Listeria infection reverses tolerance, but not when it prevents tolerance induction, may result from sub-optimal T cell priming, and ultimately the requirement of both T cell subsets for allograft rejection.

Consistent with the hypothesis that significantly more by-stander pro-inflammatory signals are necessary to reverse, compared to preventing the induction of tolerance, are the observations that the reversal of tolerance requires MyD88 signaling, while the prevention of tolerance induction by Listeria infection does not (4,64). Early MyD88-independent immune responses to Listeria infection result in IFN- β production, while a later MyD88-dependent phase regulates the production of pro-inflammatory cytokines including IL-6. The reversal of established tolerance is dependent on both IFN α R1 and IL-6 expression, in contrast to the prevention of tolerance requiring only IFN α R1 signaling and IFN- β alone being sufficient to prevent the induction of tolerance (4,64).

This observation, that the combination of IL-6 and IFN- β is able to reverse established tolerance but not IL-6 and IFN- β individually, suggests that these cytokines play non-redundant roles (4,64). Indeed, our *ex vivo* experiments reveal that IL-6, but not IFN- β , override the suppressive effects of CD4⁺CD25⁺ Tregs by promoting the activation of anti-donor proliferative responses of T cells from tolerant recipients. IFN- β , but not IL-6, enhances the frequency of IFN- γ -producing cells from tolerant mice that reject the established allograft following infection with Listeria, but not from tolerant mice that are not infected with Listeria (64). Collectively, these observations point to IL-6 and IFN- β acting in non-redundant manners, with IL-6 promoting clonal expansion of alloreactive T cells and IFN- β promoting their acquisition of effector function. Whether this is the case *in vivo*, and whether other infections or cytokine combinations can similarly abrogate established tolerance, remains to be tested.

The observations that Listeria infection can reverse established tolerance join a small list of experimental manipulations that have similar effects, including the blockade of PD-1:PD-L1 interactions (62) and the induction of mast cell degranulation (65). The understanding that significantly more by-stander pro-inflammatory signals are necessary to reverse, compared to preventing, the induction of tolerance bodes well for transplantation tolerance as a means to achieve long-term graft survival. Nonetheless, the observations that tolerance established with anti-CD154 can be reversed as a result of Listeria infection raise the possibility that non-deletional tolerance that is dependent on regulatory mechanisms may never be completely stable. When a dynamic balance between the graft-destructive and regulatory

immunity exists to maintain tolerance, it is possible that appropriate pro-inflammatory stimuli triggered by bacterial or viral infections can prompt the escape from regulation and the emergence of graft-destructive immunity (Figure 1). The definition of the infection types and the proinflammatory stimuli are therefore critical to the long-term goal of stable transplantation tolerance.

Bacterial Infections in Clinical Transplantation

Transplant recipients who are subject to life-long immunosuppression are at significantly increased risk for developing infections. Although somewhat controversial and less robust compared to the literature on viral infections, there are some clinical data in the transplantation literature that support a hypothesis that bacterial infections can enhance alloreactivity and promote allograft rejection. Infections in transplant recipients can be divided into an early, intermediate and late infectious period. The early period, defined as the first month post-transplantation, is characterized by infections seen in post-surgical patients that are not necessarily unique to transplant recipients (66-68). These include wound infections, pneumonia, urinary tract infections (UTIs), bacteremia, and vascular access- and other catheter-related infections. The intermediate time period, from the second to sixth month following transplant, is associated with opportunistic pathogens such as cytomegalovirus (CMV), *Pneumocystis jiroveci*, *Aspergillus* spp., *Nocardia* spp., *Toxoplasma gondii*, and *Listeria monocytogenes* (66-68). The infectious etiologies in the late period beyond six months post-transplantation largely mirror those seen in the greater community, and include typical respiratory viruses such as influenza, UTIs, and community-acquired pneumonia (66-68). Bacteria are important pathogens in all three time frames, with 21-62 percent of transplant recipients developing clinically significant bacterial infections (67,69).

In a recent review of the literature, Ahmed *et al.* identified a significant number of reports demonstrating bacterial infections occurring as a consequence of acute rejection, and a more modest body of literature suggesting that, in some instances, bacterial infection may precede and precipitate allograft rejection (1). In particular, successful outcomes in lung and intestinal transplantation are dampened by the high rates of rejection, chronic graft dysfunction, and frequent infections (70,71). A direct relationship between bacterial infections at large in lung transplant recipients and allograft rejection has been difficult to establish; however, several studies evaluating more narrowly defined categories of bacterial infections, especially bacterial pneumonia, have been able to demonstrate a relationship between incidence of pulmonary infections and BOS (72-74). When particular subsets of bacterial pathogens were examined, such as *Chlamydia pneumoniae* (75), *Simkania negevensis* (76), and *Listeria monocytogenes* (77), an even stronger association between infection and rejection following lung transplantation was demonstrated. In intestinal transplantation, Sigurdsson *et al.* reported that bacteremic episodes often occurred simultaneously with acute rejection or gastrointestinal post-transplant lymphoproliferative disease (71). Cicalese *et al.* suggested that in the majority (74%) of acute rejection episodes in intestinal transplantation, translocation of intestinal bacteria preceded and perhaps precipitated the rejection episode (78). In addition to the effects of transplant-localized bacterial infections on allograft rejection, there is clinical evidence supporting the possibility that bacterial infections at sites distant from the transplanted graft can also result in the generalized activation of alloimmune responses (79,80). The systemic effects of bacterial infections on alloreactivity can reflect molecular cross-reactivity between bacterial antigens and allo-specific T cell responses resulting in true allo-specific rejection. Alternatively, immune responses to bacterial infections may activate the alloreactive immune system in a bystander manner, leading to acute and chronic rejection.

Conclusions

It is apparent that data supporting a contribution of bacterial infections on allograft rejection exists, albeit they are not as robust as those for viral infections. This may be because bacterial infections tend to occur early post-transplantation when immunosuppression is typically at its highest level and likely to blunt both anti-bacterial immune responses that could otherwise enhance alloreactivity and alloresponses themselves. Further, bacterial infections can, in many circumstances, be rapidly controlled with antibiotics thereby limiting their putative effects on alloimmunity. Finally, as with viral infections, it is likely that only specific species of bacteria can enhance alloreactivity. As details become delineated on how immune responses are tailored to cope with different bacterial and viral infections, it is possible that scientists will be able to predict the impact of infections on alloreactivity and allograft fate, and ultimately identify therapeutic strategies that preserve protective anti-microbial immunity while suppressing bystander pro-inflammatory effects that enhance alloreactivity. We believe that these strategies will become increasingly important as we approach clinical protocols to minimize immunosuppression and to induce transplantation tolerance.

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Figure 1. Diagram of the balance between transplant tolerance, rejection, and the role of bacterial infection

Clinical and experimental models of transplantation show that there is a delicate balance between allograft rejection and graft acceptance. **A.** In the clinic, activation of innate and adaptive immune responses promote acute allograft rejection. Their activation by pattern recognition receptors including TLRs, NODs, and NLRs leads to alloreactive immunity, the production of proinflammatory cytokines such as IFN, TNF, IL-1, IL-6, and IL-12 that eventually lead to transplant rejection. Immunosuppression, or immunomodulation, therapy is standard treatment for transplant recipients and could lead to the inhibition of alloreactive T cells either through deletion, anergy, or the induction of suppressive, or regulatory, immunity. The overall balance between the amplitude of the pro-inflammatory versus anti-inflammatory allogeneic immune responses controls whether there will be acute rejection or stable graft acceptance. Metastable acceptance is considered in intermediate state between the two extremes. **B.** Experimental transplantation models also display the same allograft acceptance/rejection characteristics as those displayed in the clinic where rejection is controlled by the balance of pro-inflammatory versus anti-inflammatory allogeneic immune responses. In experimental models, the stability of graft acceptance is affected by the inducing tolerogenic treatment. **C.** Bacterial infections alter the balance between anti-inflammatory and proinflammatory regulatory mechanisms and either directly or indirectly, through bystander activation, promoting the alloreactive immune responses that precipitate allograft rejection.

Figure 1a. Clinical allograft acceptance reflect the dominance of anti-rejection over rejection mechanisms

Figure 1b. Experimental models of allograft tolerance reflect the dominance of tolerance mechanisms over rejection mechanisms

Figure 1c. Bacterial infections enhance rejection mechanisms and reduce the anti-rejection or tolerance mechanisms

Table 1**Pattern Recognition Receptors (PRRs)**

Source	Cellular Location	Receptor	Ligand	Role in Transplant
Toll-Like Receptors (TLRs)	Plasma Membrane	TLR1	Triacyl Lipopeptides	Unknown
	Plasma Membrane	TLR2	Peptidoglycan/Diacyl Lipopeptides	Activation prevents anti-CD154-mediated transplantation tolerance (27,28)
	Endosomal	TLR3	dsRNA	Activation prevents anti-CD154-mediated transplantation tolerance (28)
	Plasma Membrane	TLR4	LPS	Signaling may promote human lung transplant rejection (28,33,34,70)
	Plasma Membrane	TLR5	Flagellin	Unknown
	Plasma Membrane	TLR6	Diacyl Lipopeptides/Lipoteichoic Acid	Unknown
	Endosomal	TLR7	ssRNA	Unknown
	Endosomal	TLR8	ssRNA	Unknown
	Endosomal	TLR9	Unmethylated CpG DNA	Activation prevents anti-CD154-mediated transplantation tolerance (27,28)
	Plasma Membrane	TLR10	Unknown	Unknown
	Plasma Membrane	TLR11	Uropathogenic bacteria	Unknown
NOD-Like Receptors (NLRs)	Intracellular	NOD1	D-glutamyl-meso-diaminopimelic acid	Unknown
	Intracellular	NOD2	muramyl dipeptide	Lack of signaling enhances alloreactivity (38) and correlates with increased GVHD incidence and severity (39,40) or no correlation with increased GVHD incidence and severity (41,42)
Damage-associated molecular Patterns (DAMPs)	Nuclear	HMGB1	RAGE	Promotes allograft rejection (48,49)
	IntracellularM	Uric Acid (MSU)	CD14	Promotes cell damage-mediated inflammation and may participate in allograft rejection (43)
	Intracellular	Galectins	CD2 and others	Promotes cell damage-mediated inflammation and may participate in allograft rejection (44)
	Intracellular	S100 Proteins	RAGE and S100B	Promotes cell damage-mediated

Source	Cellular Location	Receptor	Ligand	Role in Transplant
				inflammation and may participate in allograft rejection (44,45)
	Intracellular	Heat shock Proteins	CD14, CD91, TLR2, and TLR4	Promotes cell damage-mediated inflammation and may participate in allograft rejection (44)