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Inflammatory triggers of acute rejection of organ allografts

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Summary

Solid organ transplantation is a vital therapy for end stage diseases. Decades of research has established that the components of the adaptive immune system are critical for transplant rejection, but the role of the innate immune system in organ transplantation is just emerging. Accumulating evidence indicates that the innate immune system is activated at the time of organ implantation by the release of endogenous inflammatory triggers. This review discusses the nature of these triggers in organ transplantation and also potential mediators that may enhance inflammation resolution after organ implantation.

Keywords

inflammation; resolution; organ transplantation

Introduction

Solid organ transplantation is a vital therapy for several end stage diseases. Although antigen-dependent immune responses orchestrated via the adaptive immune system are critical to induce both acute and chronic allograft rejection, the notion that antigen-independent injury and subsequent inflammation enhances or triggers graft rejection has gained momentum over the last few years. Knowledge gleaned from studies of brain death, sterile inflammation, and acute end organ injury have provided cross over insights to inflammation induction during organ transplantation. Our improved understanding of the signaling pathways of the innate immune system has also provided insights that are relevant to organ transplantation. This review discusses some of the triggers of antigen-independent injury that precipitate or enhance acute allograft rejection. Mediators that regulate the resolution of inflammation and may influence the outcome of organ transplantation will also be considered.

Antigen-independent injury occurs prior to implantation in the recipient Organ transplants are unique in that they are subjected to various forms of antigen-independent injury (Fig. 1). Specifically, an organ undergoes injury during brain death in the donor, the ischemia of organ harvest, and the subsequent reperfusion injury that occurs with restoration of blood

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during organ implantation in the recipient, a condition known as ischemia reperfusion injury (IRI).

Comparison of the outcomes between organs from living-related donors and organs from brain dead donors has indicated that brain death has detrimental effects on allograft function (1–4). Importantly, most organs are harvested from brain dead donors, and for certain organs, such as cardiac transplants, this is the sole source of donors. Although an area that still requires much investigation, experimental and clinical studies have provided some insights of the mechanisms of how brain death can influence subsequent organ function. Studies have shown that brain death upregulates inflammatory cytokines and chemokines within the transplant including the production of interleukin-1β(IL-1β), IL-6, tumor necrosis factor-α(TNF-α), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon-γ(IFN-γ), and monocyte chemotactic protein-1 (MCP-1) (1, 5, 6). Additionally, brain death enhances the expression of major histocompatibility complex (MHC) class II, and adhesion molecules (e.g. E-selectin) (7). The upregulation of all these mediators could increase the ability of the graft vasculature to present antigens to circulating T cells that attach to the graft. Furthermore, the changes induced by brain death may promote the recruitment of innate immune cells, predominantly neutrophils, to attach to the graft vasculature. These experimental studies of brain death are supported by clinical observations in which kidney transplants from brain dead donors exhibit evidence of increased macrophage and T-cell infiltration as compared to kidney transplant from living related donors (8). Brain death also activates the complement system within the graft. Specifically, experimental work in rodents has demonstrated that the central complement mediator, C3a, is deposited within hearts during brain death and that brain death-induced inflammation within cardiac allografts is C3a dependent (9). Recently, a rodent study provided evidence that inhibition of the complement system within the heart transplants reduced inflammation that resulted from brain death (10). Furthermore, complement inhibition enhanced allograft survival in a major histocompatibility mismatched heart transplant model (10, 11). In summary, brain death induces a variety of phenotypic changes that result in enhanced inflammation within a transplant, which have the potential to compromise graft function after implantation. However, the molecular mechanisms by which brain death mediates these effects have not been fully elucidated. Possibilities include inflammation activation via the nervous system, release of humoral factors, and impaired homeostasis.

Most cadaveric organs are exposed to cold storage in transit to the transplant center for subsequent implantation into the recipient. Clinical studies have shown that prolonged ischemia time has a detrimental effect on the outcomes after transplantation (reviewed in 12). The exact mechanisms by which prolonged ischemic time leads to worse outcomes after transplantation are yet to be fully elucidated, but experimental studies have provided information that increasing ischemic time or hypoxic injury enhances both humoral and T-cell responses to vascularized grafts. In these studies, human coronary arteries were implanted into immune deficient mice that were either intravenously transfused with human antibodies or human peripheral blood mononuclear cells, respectively (13, 14). It is possible that increasing hypoxic insult leads to the upregulation of factors that promote deposition of antibodies, or enhance T-cell attachment to the graft vasculature. While the mechanisms by which cold storage and graft hypoxia increase the immunogenicity of the allograft remain to be fully elucidated, there are ongoing clinical efforts to reduce the impact of cold storage on graft function. Such efforts include approaches to perfuse organs *ex vivo* with normothermic solutions and relevant nutrients (reviewed in 15).

Peri-operative antigen-independent injury to an organ

When an organ is implanted in a recipient, blood flow is immediately restored and this exacerbates the injury that has already occurred as a result of brain death, organ preservation, and hypoxia. The resulting IRI leads to graft inflammation and localized activation of the innate immune system. IRI is already recognized as a central feature of several medical disorders including acute coronary syndrome, stroke, cardiac arrest, and trauma (16).

IRI is characterized by a rapid (i.e. within minutes to hours) infiltration of neutrophils to the site of injury, in response to increases in specific chemokines (e.g. IL-8), with subsequent recruitment of other cells of the immune system (e.g. inflammatory macrophages) (16). In organ transplantation, the procurement, harvest, and implantation are conducted under stringent sterile conditions in an operating theater. Any evidence of infection within the donor results in the exclusion of the donor. Some organs such as intestines and lungs have commensal flora and it is possible that these may influence inflammation after implantation. But organs transplants such as kidney and hearts do not. Thus, the inflammation that is a result of IRI during heart or kidney transplantation is likely to be sterile. This contrasts with the inflammation that occurs with crush injury and trauma in which contributions from environmental activators of inflammation, including contaminating microbes, may occur.

Sterile inflammation is a concept that has gained increasing appreciation over the last few years (17–19). Although studies with pathogens have provided key insights as to how signaling of the innate immune system occurs (20), it has become clear that inflammation occurs without microbes. Conceptually, necrotic cell death leads to the disruption of cell membranes and tissue barriers within the transplant. The subsequent release of intracellular contents and components of the extracellular matrix occurs, which are typically hidden from the immune system under quiescent conditions, activate the innate immune system. Given that vascularized organ transplants are implanted under sterile conditions as discussed above, the primary activators of the innate immune system after organ transplantation are highly likely to be endogenous (and thus not microbial in origin). Endogenous innate immune activators have been considered in a variety of experimental models and clinical diseases (17). Thus, a definition of a primary trigger of inflammation in organ transplantation is a substance that is released during the sterile injury of organ implantation and is either sufficient to induce inflammation or synergizes with another factor to promote activation of the innate immune system (19). Experimental approaches to investigate any potential endogenous factor that triggers inflammation after organ transplantation, either *in vivo* or *in vitro*, should demonstrate that the factor is not contaminated with microbial motifs [e.g. lipopolysaccharide (LPS)]. With this definition, microbes would not be considered primary activators of inflammation that occurs after organ implantation, although microbes can influence graft function via a variety of mechanisms as discussed later.

Endogenous triggers of sterile inflammation relevant to organ transplantation

The endogenous triggers of sterile inflammation that are relevant to organ transplantation can be categorized into intra and extracellular origins (Table 1, Fig. 2). Intracellular activators include nuclear proteins and cellular chaperones, mitochondrial components, and uric acid. High mobility group box 1 (HMGB1) is a nuclear protein that binds DNA and enhances gene transcription. Increased levels of HMGB1 correlate with increasing end organ injury in a variety of experimental models of kidney, cardiac, hepatic IRI, and islet transplantation (21–25). Pharmacological approaches that either limit nuclear translocation of HMGB1 or employ specific blocking antibodies reduce inflammation in renal IRI murine

models (21, 26, 27). The ability of anti-HMGB1 blocking therapy to reduce renal IRI is dependent on the presence of Toll-like receptor 4 (TLR4) (28). Studies in hepatic IRI models have also found that inflammation is dependent on this TLR (29). These studies imply that HMGB1 has complex cellular and intracellular interactions. The release of intracellular HMGB1 may engage TLR4 to induce inflammation. Additionally, increased intracellular HMGB1 may translocate to the nucleus (independently of TLR4) and enhance inflammation. In addition to TLR4, another known receptor for HMGB1, the receptor for advanced glycation products (RAGE) has also been shown to promote transplant rejection in models of lung and liver IRI, as well as islet and heart transplantation (25, 30–32). Furthermore, administration of recombinant HMGB1 to disease free mice elicits a systemic inflammatory response in healthy mice, providing *in vivo* evidence that HMGB1 is sufficient to induce inflammation (21). Hence, these studies indicate that HMGB1 acts as an inflammatory trigger in IRI models. However, these warm IRI models have not accounted for the effects of donor harvest and organ implantation.

One report employed an antagonist to HMGB1 to show that this delayed the tempo of acute cardiac allograft rejection in mice by one week (33). This study did not subject the allograft to cold preservation prior to implantation. The delay in allograft rejection was associated with reduced intra-graft TNF-α levels. Another experimental study that employed a syngeneic heterotopic heart transplant model in mice and subjected the allograft to 8 h of cold ischemia prior to implantation demonstrated that HMGB1 inhibition reduced IRI and IL-17A levels, a cytokine that enhances neutrophil migration to sites of inflammation (22). A human study demonstrated that cadaveric renal transplants exhibit higher levels of HMGB1 than living related donor kidneys (34). Furthermore, this study provides evidence that HMGB1 signals via TLR4 to induce *in vitro* inflammatory responses (34). The focus on TLR4 is important, as clinical studies have shown that humans with a polymorphism of TLR4, which reduces signaling of this receptor, have a delay in onset to acute and chronic allograft rejection (35, 36). Overall, there is strong experimental support that HMGB1 may be an inflammatory trigger in organ transplantation.

Heat shock proteins (HSPs), which act as cellular chaperones, can similarly activate cells of the innate immune system such as macrophages and monocytes via TLR4 (37, 38). While the concentration of HSPs is raised in experimental models of IRI and human kidney transplants (39), it is not yet clear if HSPs act as inflammatory triggers that precipitate acute transplant rejection. Although one experimental study found that absence of donor HSP-70 slightly delayed allograft rejection, the study did not provide evidence that purified HSPs induced inflammatory responses [i.e. stimulation of dendritic cells (DCs) to produce inflammatory cytokines] (40). Additionally, a later study failed to detect HSPs in rejecting skin lysates and also failed to show that purified HSP-70 activates DCs (41). Importantly, rejection in a minor mismatched skin transplant model, which has been previously shown to be MyD88-dependent (MyD88 is an adapter signal downstream of all TLRs except TLR3 and downstream of IL-1 and IL-18 receptors) (42), is not dependent on HSP-70 in either donor or recipient (41). Furthermore, one study found that a different form of HSP, gp96, administered onto skin transplants, enhances transplant survival in a minor mismatch model, indicating that HSP may exhibit protective effects (43) and others have suggested that HSP may enhance ischemic preconditioning to reduce IRI (44). Hence, the role HSPs play in organ transplant is complex and differences between studies may reflect different forms of HSP studied and their diverse biological effects.

Components of the extracellular matrix have been associated with the development of acute and chronic allograft rejection (45, 46). Hyaluronan (HA) is a glycosaminoglycan produced by mesenchymal cells that is in a high molecular weight form in quiescent states. However, during the disruption of the extracellular matrix with inflammation, HA fragments into

lower molecular weight forms. How this occurs in not completely clear, but it is possible that tissue trauma disrupts the extracellular matrix to fragment HA, alternatively reactive oxygen species from IRI may fragment HA. Regardless of how HA is fragmented, prior work has indicated that these lower molecular forms activate the innate immune system (e.g. macrophages or DCs), via TLR2 and TLR4 (47–49), whereas the higher molecular weight form enhances immune regulation (e.g. enhancing the function of regulatory T cells)(50). HA can also engage the CD44 receptor on T cells. Low molecular weight HA may inhibit the interaction between CD44 and T cells, via unclear mechanisms, in addition to their proinflammatory effects on innate immune cells. For example, experimental studies have investigated how intravenous infusion of low molecular HA impacts the tempo of acute and chronic allograft rejection. These studies found that in combination with cyclosporine, low molecular weight HA delays the onset of allograft rejection in both kidney and cardiac murine models (51, 52). However, the biological relevance of administering low molecular weight forms of HA remains uncertain. In contrast, local release of fragmented (and thus low molecular weight) HA within the transplant after implantation may promote inflammation and impair graft function. Such a concept has yet to be tested experimentally due to a lack of tools (i.e. mice in which HA is genetically deleted at sites of inflammation). In summary, HA is associated with acute and chronic graft rejection, but its effect are complex, possibly due to studies employing different molecular weight forms. The precise role of HA in organ transplantation awaits definitive studies.

There are other known triggers of sterile inflammation that have not yet been causally linked to acute or chronic rejection of solid organ allografts. Mitochondrial components, including DNA and peptides, have been implicated in promoting inflammation during trauma. Mitochondria may have arisen from intracellular symbiotic bacteria. Thus, it is appealing to consider that with cell necrosis, released mitochondrial components could activate similar innate immune receptors and signaling pathways as invading pathogens. A clinical study of trauma patients demonstrated that mitochondrial DNA is released into the circulation (53). Furthermore, mitochondrial peptides activate formyl peptide receptor 1 (FPR1) and TLR9, innate immune receptors that both provide host defense to microbes, to promote inflammation (53, 54). Other mitochondrial components, e.g. adenosine triphosphate (ATP), may promote inflammation. ATP signals via a purinergic receptor PX27, in combination with TLR activation, to induce inflammation via the inflammasome; an intracellular multi-protein complex that transduces a variety of inflammatory signals (55). However, studies mechanistically linking mitochondrial components and inflammation induction after organ transplantation have not yet been documented.

Uric acid is the product of purine metabolism and produced by a wide variety of cells. During necrosis, the increased uric acid concentration promotes formation of monosodium urate (MSU) crystals after exposure to extracellular sodium. MSU stimulate DCs *in vitro* by upregulating costimulatory molecules and triggers the release of proinflammatory cytokines such as IL-1β and TNFα (56), indicating that MSU acts as an inflammatory trigger *in vitro*. Additionally, direct injection of MSU into the peritoneum of mice induces neutrophil recruitment *in vivo*, an inflammatory response that is abrogated in IL-1R $^{-/-}$ and myeloid differentiation factor 88 (MyD88)$^{-/-}$ mice (57). Uric acid also activates the NLPR3 inflammasome to induce IL-1β production (58). Although there is a clinical association between higher uric acid levels and worsen kidney transplant outcome (59), the mechanistic role uric acid may play in organ transplantation is yet to be elucidated. Furthermore, the mechanistic role the inflammasome plays in acute and chronic allograft rejection also remains unclear, although experimental studies have linked inflammasome activation and acute cardiac allograft rejection (60) and graft function in lung transplantation (61).

Studies of inflammatory triggers in organ transplantation have typically employed a candidate approach, testing the role of a specific activator based upon its known function. However, it is conceivable that several triggers contribute to inflammation after organ implantation, and non-biased approaches will likely yield novel information regarding inflammation induction in organ transplantation. We recently adapted an *in vitro* assay in which DCs are cultured with necrotic material to show that the process of skin transplantation augments the inflammatory response of DCs (62). After we documented that proteins are major contributors to inflammatory response of DCs, we performed comparative proteomics between non-transplanted and syngeneic skin transplants, and non-transplanted grafts and allogeneic skin transplants (62). Our analysis revealed that the protein haptoglobin was upregulated after either syngeneic or allogeneic skin transplantation, a result we validated with an independent enzyme-linked immunosorbent assay (ELISA) (62). *In vitro* cultures demonstrated that haptoglobin activates DCs via MyD88. We also employed haptoglobin deficient skin transplants in a minor matched transplant model, which we had previously employed to demonstrate that graft rejection is MyD88-dependent (42), to show that haptoglobin within the donor accelerates the tempo of acute graft rejection (62).

Haptoglobin is an acute phase protein that binds free heme, enhances heme uptake by macrophages, and prevents free heme from inducing oxidative stress (63, 64). Haptoglobin is synthesized in the liver but is also produced in the lung, skin, and kidney, particularly in the context of inflammatory disorders, e.g. psoriasis or acute ischemia (65, 66). Besides its heme-binding properties, haptoglobin can alter inflammation and immunity. For example, it inhibits LPS-induced inflammatory responses by macrophages (67), decreases granulocyte chemotaxis (68), and reduces inflammation in experimental autoimmune encephalitis (69). Yet, haptoglobin can enhance monocyte chemoattraction (70) and augment adaptive immunity to nominal antigens (e.g. delayed type hypersensitivity responses) (71). Thus, haptoglobin exhibits immune altering properties, which are largely anti-inflammatory. Our recent report indicates that haptoglobin also exhibits proinflammatory properties (62).

In humans, the haptoglobin gene is encoded by two alleles, Hp1 and Hp2, with three resulting genotypes: homozygous Hp1/Hp1, Hp2/Hp2 and heterozygous Hp2/Hp1. Individuals with the Hp2/Hp2 phenotype exhibit larger acute myocardial infarctions, especially when diabetes is present, than Hp1/Hp1 individuals (63, 72– 75). Myocardial infarction is a manifestation of atherosclerosis, a disease in which the innate immune system plays a role in pathogenesis (74, 76). The Hp2/Hp2 genotype has also been correlated with enhanced graft vs. host disease after allogeneic bone marrow transplantation as compared to the other genotypes (77). Although it is not clear if different haptoglobin genotypes lead to different haptoglobin levels, the Hp2/Hp2 form encodes a protein of higher molecular mass than the Hp1/Hp2 form, which binds free heme less efficiently and leads to increased oxidative vascular stress (78). Haptoglobin induction within the renal cortex has recently been correlated with acute kidney injury (66), another condition in which the innate immune system contributes to pathogenesis (79). A prior clinical report indicates that polymorphisms in haptoglobin correlate with chronic allograft rejection after cardiac transplantation and liver transplantation (80, 81). Our prior report (62) in a minor mismatch transplant model provides evidence that donor haptoglobin enhances transplant rejection. However, it will be important to discern the role haptoglobin plays in more immunogeneic experimental transplant models.

Role of cytokines to trigger inflammation after organ transplantation

Bioactive cytokines are released from necrotic cells during sterile inflammation. For example, IL-1α is released from necrotic cells and sensed by the IL-1 receptor to enhance

neutrophil migration via the production of chemokines (e.g. CXCL1) in mesothelial cells (82). This was demonstrated in a murine sterile peritonitis model, in which inflammation was induced by injection of MSU crystals into the peritoneum, with IL-1 inhibition via an inhibitory antibody or injection of MSU in IL-1 receptor$^{-/-}$ mice reducing neutrophil recruitment into the peritoneum (83). In an experimental transplant model in which a human coronary artery is implanted into an immunodeficient mouse, which is reconstituted with allogeneic human peripheral blood mononuclear cells (84), IL-1α enhances anti-donor adaptive immunity. In particular, IL-1α is present in endothelial cells lining the human artery and inhibiting IL-1α reduces subsequent T-cell graft infiltration (84). This study also indicates that sterile inflammatory pathways may enhance subsequent anti-donor adaptive immunity.

Other cytokines such as TNF-α modify early inflammatory responses after transplantation. For example, one study found that gene expression of TNF-α was expressed within the transplant early after cardiac transplantation in mice, although the hearts were not subjected to cold storage and ischemia (85). The study also found that inhibition of TNF-α reduces neutrophil migration into cardiac transplants and decreases histological evidence of inflammation (85). While the source of TNF-α in this experimental study was not addressed, it is possible that an endogenous trigger released during IRI of organ implantation led to the production of TNF-α by innate immune cells (e.g. DCs or macrophages) or non-hematopoietic cells (e.g. vascular cells) within the transplant. Another study found that TNF-α synergizes with IL-6 to prevent transplant tolerance to skin allografts (86). These cytokines increase T effector cell proliferation, rendering these cells less susceptible to immune regulation (86). This study suggests the cellular source of TNF-α and IL-6 is from DCs, although this was not directly shown *in vivo*.

The importance of identifying the inflammatory triggers of organ transplant rejection, and strategies to identify them

Many endogenous inflammatory triggers of transplant rejection are still to be discovered. The importance of identifying these triggers is the potential to inhibit them at the site of injury (i.e. within the transplant) rather than systemically. In organ transplantation, there is a unique ‘window of opportunity’ to treat the organ during the harvest and procurement procedure and prior to implantation into the recipient (Fig. 1). Such a strategy could allow decorative treatment of the graft and avoid systemic treatment of the recipient. As mentioned above, several endogenous ligands share similar signal transduction pathways as microbial motifs. For example, HMGB1 signals via TLR4, and HA employs TLR2 and TLR4 with subsequent signaling via MyD88. These TLRs are activated by Gram positive and negative bacteria, respectively (87). Several endogenous triggers, e.g. UA and asbestos, activate the inflammasome (88, 89). Although the mechanistic role of the inflammasome in organ transplantation has not yet been established, this signaling pathway is also activated by a variety of pathogens [e.g. influenza virus (90)]. Therefore, a strategy that targets the innate immune system via systemic inhibition to reduce inflammation in organ transplantation will render the host susceptible to infection (i.e. primary or re-activation of latent pathogens), posing an additional risk to transplant recipients who will receive generalized pharmacological immune suppression. Importantly, patients with rheumatoid arthritis or inflammatory bowel disease who receive systemic anti-TNFα therapy are at increased risk of infection (e.g. mycobacterium).

Non-biased, exploratory approaches in relevant models (i.e. tissue from transplants) will be required to identify novel inflammatory triggers of organ transplant rejection. Such approaches will include trancriptome analysis (e.g. deep sequencing), lipidomics, and proteomics. Potential targets that are differentially regulated by organ transplantation could

then be purified and tested *in vitro* systems (i.e. assessment of whether a candidate triggers an inflammatory responses by DCs or macrophages), ideally with the capability of high throughput data acquisition and analysis. Positively identified targets could then be investigated with in vivo transplant models via the generation of relevant genetic or pharmacological approaches. Integration of data from investigative screens by bioinformatic and systems biological analysis may yield information concerning which targets are potential major regulators of inflammation after organ transplantation. Potential targets could then be assessed to determine if they are druggable and if they are present in human transplants.

Influence of infections and solid organ transplant rejection

Clinical studies several decades ago made the association between viral and bacterial infections and worse outcomes after organ transplantation (91). Additionally, organs that are colonized with commensal bacteria such as skin, lung, and small intestine characteristically exhibit faster tempo of acute graft rejection and resist transplant tolerance (defined as the ability of a recipient to accept an allograft without chronic immune suppression and the ability to respond to third party antigens) as compared to organs that do not have commensals (e.g. kidney and heart transplants) (92). Although it is tempting to attribute the presence of commensal bacteria as causally related to this phenomenon, the mechanisms as to why these organs exhibit an enhanced rejection kinetics are likely to be complex and not yet fully clarified. Importantly, germ-free mice reject germ-free skin allografts of varying degrees of immunogenicity, indicating that the presence of skin commensals are not essential for skin graft rejection (92, 93). As stated above, clinical organ transplantation induces a sterile inflammatory response. Thus, we consider that the principle triggers of inflammation after organ transplantation likely to be endogenous rather than microbial in origin.

Although data demonstrating that the primary driver of inflammation after organ transplantation is not microbial, infections are known to influence transplant rejection via multiple mechanisms (reviewed in 94). Specifically, prior infections (e.g. herpes viral infections) lead to the accumulation of memory T cells with cross reactivity to alloantigens, a phenomenon known as heterologous immunity. Prior experimental studies have shown that the presence of such T cells accelerates the tempo of acute transplant rejection and impairs the induction of transplantation (95). It is also possible, but not yet tested, whether altering the commensal flora in a recipient impacts graft reactive immune responses. Experimental studies with systemically administered bacterial pathogens, e.g. *Listeria monocytogenes* or *Staphylococcus aureus*, have demonstrated that these pathogens can impair the induction of transplantation tolerance to skin and cardiac allografts (96–98). In the case of *Listeria* infection impaired induction of transplant tolerance is dependent on type I interferon (IFN) signaling but is MyD88 independent (96). Yet MyD88 is required to break tolerance to accepted allografts induced by *Listeria* infection (97). Why MyD88 is dispensable to impair transplant tolerance induction but is required to break established tolerance with infection with the same pathogen is unclear. With *Staphylococcus* infection, MyD88 signaling is also required to impair transplant tolerance, an effect that is IL-6 dependent (98), although the cellular source of this cytokine has not been determined. In these experimental studies, bacterial infection enhances the production of anti-donor effector T cells that either escape the effects of the immunomodulatory protocol (e.g. costimulatory blockade) or are re-activated by inflammation induced by the microbe (i.e. bystander inflammation). Hence, these experimental studies provide evidence that bacterial infections induce inflammation that alters the fate of organ transplants, specifically the immune regulation of allografts. Whereas there is clear evidence that bacterial-induced inflammation activates graft-reactive T cells, it is possible that concurrent bacterial infection may also

induce organ injury and the release of endogenous activators, further enhancing inflammation and provoking graft rejection. Ultimately, mechanisms by which concurrent infections enhance solid organ transplant rejection are likely to be complex and are not yet fully elucidated.

Mediators of inflammation resolution in organ transplantation

For an organ to revert back to a quiescent state after an inflammatory insult, inflammation must resolve. Inflammatory triggers activate several innate immune signaling pathways including pathways downstream of the TLRs (e.g. via MyD88 and Trif) and the IL-1 receptor (also via MyD88). These pathways include feedback negative signal transducers (via negative regulators including IRAK-M and TIR/SIGIRR), which dampen the inflammatory response. Indeed, experimental studies in which these negative regulators are deleted in mice have shown their importance in inflammatory regulation in a variety of models [e.g. sepsis injury, transplant tolerance (86, 99), autoimmune myelitis](100, 101). Regarding experimental transplantation, a murine kidney transplant model in which allografts are spontaneously accepted found that absence of TIR8 in the donor induces acute allograft rejection (99). Absence of donor TIR8 is associated with increased IRI after graft implantation. In a murine skin allograft model, absence of IRAK-M in the recipient increases IL-6 and TNF-α levels and impairs the ability of costimulatory blockade to enhance transplant survival (86).

One of the first cellular mediators of sterile inflammation are neutrophils, which migrate to inflammatory sites within hours of the initiation of injury to form clusters, as was recently demonstrated by dynamic *in vivo* imaging in murine models of cardiac IRI and burn injury (102, 103). Neutrophils are short-lived cells that propagate the initial inflammatory response. They are vital in host defense against infection, interacting with cells of both the innate and adaptive immune system (104). Effective resolution of inflammation requires the cessation of further neutrophil trafficking to the inflammatory site and either the efficient clearance of extravasated cells undergoing apoptosis at the site of acute inflammation, or fugetaxis (reverse migration) of viable cells (105). Important mechanisms in this regard include the secretion of mediators (e.g. annexin A1) by apoptotic neutrophils. Moreover, neutrophils upregulate signals (e.g. sphingosine 1-phosphage) that increase engulfment by macrophages, a process termed efferocytosis (106).

Infiltrating monocyte-derived macrophages are initially proinflammatory but are capable of changing their phenotype to promote resolution. A transcriptional analysis comparing macrophages purified during the inflammatory or resolution phase of murine peritonitis determined a dynamic shift in cell profile, with resolution-phase macrophages upregulating genes including TIM4 and TGFβ, which are involved in tissue repair and clearance of inflammatory cells (107). Multimodal facilitation of resolution and a return to tissue homeostasis is achieved by production of vascular endothelial growth factor (VEGF), which increases tissue angiogenesis and restoration of oxygen to injured tissue and the release of both proresolving lipid mediators and immunosuppressive cytokines (e.g. IL-10), which may allow for recruitment of regulatory T cells that further assist in inflammation resolution (108).

Resolution of inflammation is not merely a passive inhibition of inflammatory pathways or clearance of inflammatory cells. Initial mediators that are released at sites of injury such as eicosanoids are proinflammatory and amplify the inflammatory response. Eicosanoids are derived from arachidonic acid via cyclooxygenase enzymes. Prostaglandin E2 (PGE2) is an initial promoter of inflammation but is known to enhance resolution partly by inducing a class switch to mediators that actively promote the resolution process or via the induction of

immune suppressive cytokines such as IL-10 (108, 109). A study in a murine cardiac allograft model found that a PGE2 agonist enhanced cardiac allograft survival by one week and was associated with reduced inflammation and reduce inflammatory gene expression within the allograft, although this study did not determine if PGE2 elevated resolution mediators (110). However, the therapeutic utility of enhancing PGE2 will need to be balanced with prior experimental data in syngeneic bone marrow transplant model showing that PGE2 can increase susceptibility to bacterial pneumonia (111).

Most resolution mediators are lipids and metabolized from the arachidonic acid pathway by lipoxygenase (lipoxins) or synthesized from essential omega-3 polyunsaturated fatty acids (resolvins and protectins) [together termed specialized pro-resolving lipid mediators (SPM), reviewed in (108, 112)]. These mediators can be produced by immune cells (e.g. neutrophils, eosinophils, and macrophages) but also by the vasculature and exhibit considerable overlap in their action, enhancing several resolution pathways (113). Lipoxins act on specific receptors, which result in reduced neutrophil migration by enhancing cellular arrest (108). Lipoxins also exhibit anti-fibrotic properties, which may prevent pathological chronic inflammation.

The role of lipid mediators of resolution in organ transplantation is yet to be fully clarified. A study that employed both clinical lung transplant samples and experimental models of cardiac and kidney transplantation, demonstrated that lipoxin A_4 is present within the bronchiolar lavage of patients that exhibit acute allograft rejection, the concentration correlating with severity of clinical pathology. Given its known bioaction, the authors describe this associated rise as counter-regulatory, aimed at ameliorating neutrophil-induced tissue damage. Accordingly, transgenic recipient mice overexpressing human lipoxin A_4 receptors exhibit a slightly delayed time to cardiac allograft rejection with reduced neutrophil graft infiltration (114). Additionally, systemic treatment with resolvin E1, a further SPM, enhances kidney allograft survival in mice (114). However, the specific mechanisms by which these mediators delay graft rejection, and whether the small delay in graft survival induced by each mediator could be enhanced when administered with other immunomodulatory therapies, have not been addressed. Importantly, whether SPM reduce chronic vasculopathy, the major cause of solid organ transplant loss, has not yet been determined. A critical distinction to resolving inflammation in organ transplantation is that the foreign tissue persists, whereas with an infection the pathogen is typically cleared (with the exception of chronic or latent infections). Further examination as to how resolution mediators are induced within a transplant and the mechanisms by which they could promote allograft survival may provide additional treatment options to limit the effects of inflammation induced after organ transplantation. Any approaches seeking to augment inflammation resolution in organ transplantation must first determine the effects of this approach on host defense to infection (115).

Conclusions

The mechanisms that lead to inflammation induction after implantation of solid organ transplants are only beginning to be elucidated. Identification of the triggers of inflammation after solid organ implantation may lead to novel therapeutics to reduce inflammation within the transplant without disabling systemic host defense pathways that are required to defend against infection. Organ transplantation offers a unique ‘window of opportunity’ in which the transplant can be treated prior to implantation into the recipient. Most investigation into the inflammatory triggers of organ transplantation are based on prior studies in which a potential trigger’s function is known from prior studies in non-transplant models. Such biased approaches are unlikely to discover the majority of inflammatory triggers of organ transplantation. As it is likely that most of the inflammatory triggers have not yet been

discovered, non-biased discovery approaches will be required to identify critical inflammatory triggers in organ transplantation.

Inflammation resolution in solid organ transplantation is a largely unexplored area. Determining the factors that promote inflammation resolution may allow for limitation of deleterious inflammation that occurs in organ transplantation. Novel approaches to enhance resolution should avoid increasing susceptibility to infection. Approaches to effectively inhibit local inflammation within organ transplants coupled to strategies to enhance inflammation resolution may maximize the effectiveness of solid organ transplantation, a critical therapy for several end organ diseases.

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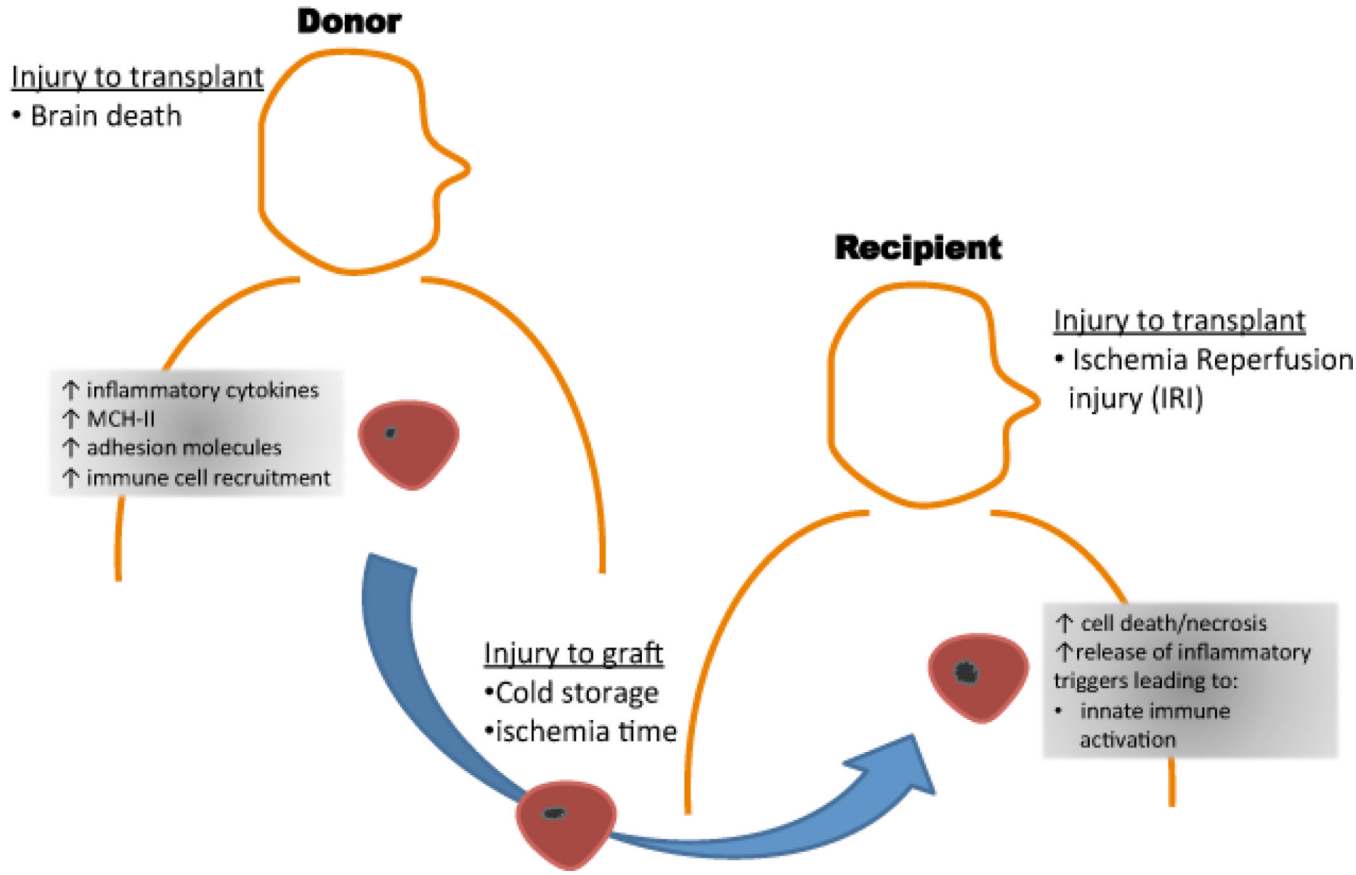

Fig. 1. The process of organ harvest and implantation induces injury in the transplant

Changes to an organ occur at the time of brain death where a series of inflammatory changes occur within the organ. The procurement and transit of the organ imparts a further ischemic injury, which is exacerbated at the time of implantation when reperfusion exacerbates the injury to the transplant (known as ischemia reperfusion injury). Injury to the transplant leads to the release of inflammatory triggers that are sensed by immune and non-immune cells to initiate the inflammatory response to the transplant.

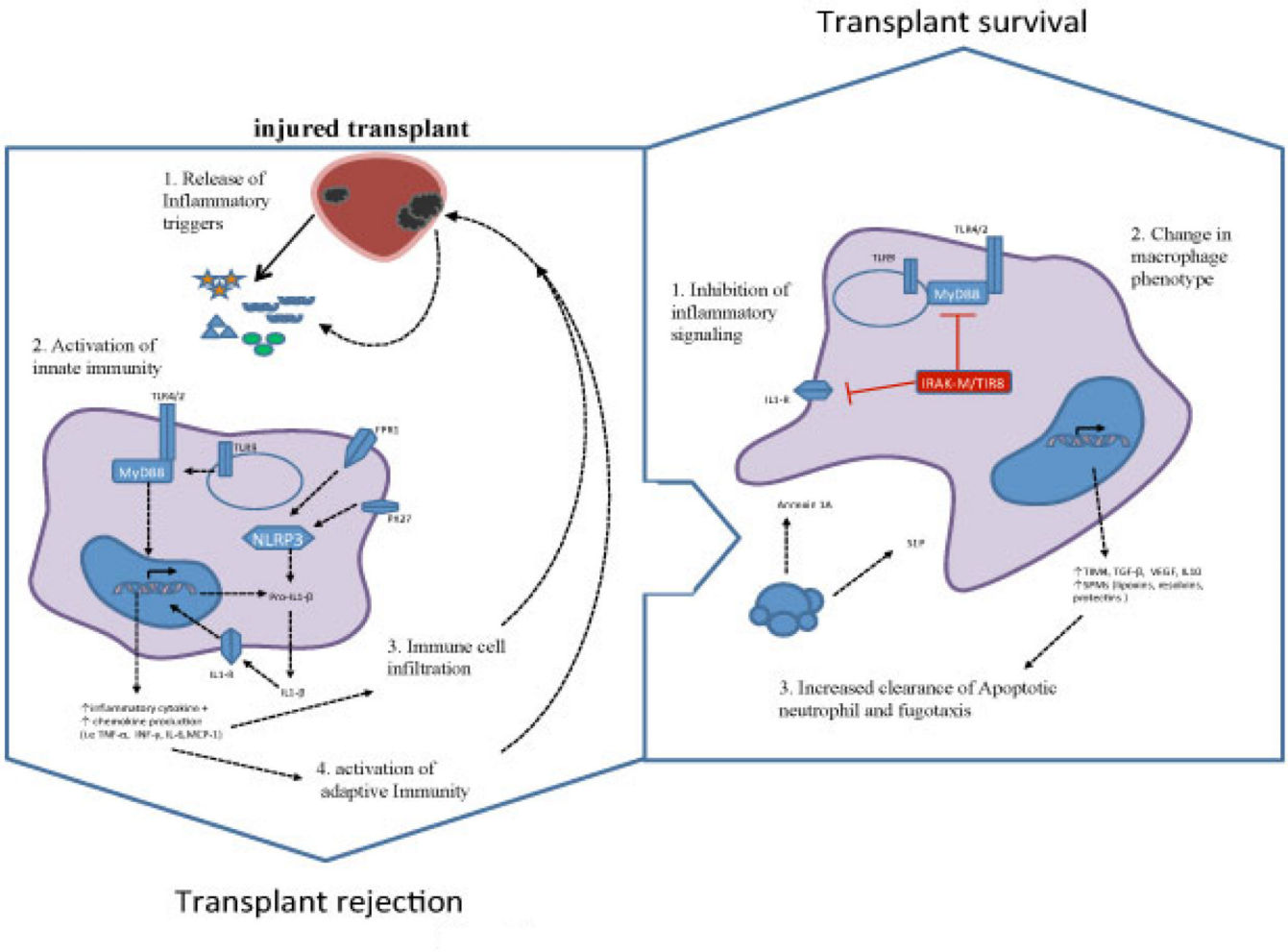

Fig. 2. The balance of initiation of inflammation and inflammation resolution after organ implantation

The release of inflammatory triggers within the organ induces inflammation. Several inflammatory triggers have been identified as inducing inflammation after organ transplantation (e.g. HMGB1)(Table 1). These triggers are sensed by immune cells (e.g., macrophages and DCs) and non-immune cells (e.g. epithelial cells) and transduce inflammatory signals via a variety of pathways that are downstream of the TLRs and inflammasome. Many inflammatory triggers released after organ transplantation are likely yet to be identified, and the inflammatory pathways that induce inflammation after organ transplantation are still to be fully elucidated. Several pathways are activated after organ transplantation that inhibit further inflammation. Furthermore, certain immune cells (e.g. macrophages) many change their phenotype to one of resolution by secreting mediators that enhance the clearance of apoptotic cells and impair further recruitment of inflammatory cells into the transplant.

Table 1

Triggers of sterile inflammation in solid organ transplantation

Trigger	category	Innate immune sensor(s)	Relevant reports	references
Primary triggers of innate immune activation in organ transplantation				
High mobility group box 1 (HMGB1)	intracellular	TLR-4, TLR-2, RAGE	- HMGB1 levels in acute rejection in kidney, heart, liver and islet models. - proinflammatory cytokines (TNF-α,IL-1b, IFN-γ,IL-12,IL-6, IL-17) - increased chemokines (MCP-1, KC,IP-10) - immune cell infiltration - HMGB1 blocking/ neutralization promotes graft survival	21– 2534 21–26, 30, 33 21, 26 26, 32 26–28, 30 32, 33
Hyaluronan (HA) (Fragmented or low m.w.)	extracellular	TLR2, TLR4, MyD88, TIRAP, CD44	- fHA in rejected human kidney grafts - Promotes DC mediated priming of allogeneic T cells - DC maturation and chemokine secretion, TNF- α production - Promotes Treg function (high m.w. HA)	45 46 46, 47, 48 50
Haptoglobin	Extracellular (secreted)	MyD88	- levels in syngeneic or allogeneic skin transplants - Promotes DC maturation; IL-6, TNF-a, IFN-g and priming of allogeneic T cells - immune cell recruitment - Enhance adaptive immune responses	62 62 70 71
Putative triggers of innate immune activation in organ transplantation				
Heat shock proteins (HSP)	intracellular	TLR-4, CD91	- Activation of monocytes/ macrophges - HSP levels in liver IRI models - HSP-70 increases graft rejection - HSP-70 not an innate immune trigger in skin transplant model - Gp96/HSP promotes graft survival, ischemic preconditioning	37, 38 39 40 41 43, 44
Uric acid	intracellular	NLRP3 inflammasome	- Uric acid levels associated with kidney graft rejection	59
Mitochondrial components (DNA, f-peptides, ATP)	intracellular	FRP1, TLR9, Px27, NLPR3 inflammasome	Mechanistic role yet to be determined in organ transplantation	