Novel Soluble Mediators of Innate Immune System Activation in Solid Allograft Rejection

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Abstract. During the past years, solid allograft rejection has been considered the consequence of either cellular- or antibody-mediated reaction both being part of the adaptive immune response, whereas the role of innate immunity has been mostly considered less relevant. Recently, a large body of evidence suggested that the innate immune response and its soluble mediators may play a more important role during solid allograft rejection than originally thought. This review will highlight the role of novel soluble mediators that are involved in the activation of innate immunity during alloimmune response and solid allograft rejection. We will also discuss emerging strategies to alleviate the aforementioned events. Hence, novel, feasible, and safe clinical therapies are needed to prevent allograft loss in solid organ transplantation. Fully understanding the role of soluble mediators of innate immune system activation may help to mitigate solid allograft rejection and improve transplanted recipients' outcomes.

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INTRODUCTION

The role of innate immunity in solid organ transplantation has been poorly studied in the past, but it is now generally accepted that it represents a very early step in allograft rejection that may guide to the development of adaptive immune response. This is extremely important in the light of the fact that immunosuppressive drugs have efficiently contributed to halt the activation of the adaptive immune response without affecting the innate immune system.[1] Several experimental and clinical data sustain the idea that the injury to the donor organs occurring during/after transplantation in the recipient may sensitize and trigger the innate immune response, thus initiating the alloimmune response.[2] Indeed, innate immune responses can be triggered on as a consequence of antigen-independent stimuli, such as ischemia/reperfusion, surgical injury, inflammation, tissue destruction, cellular damage, systemic stress, brain death, and microbial infections occurring at the time of transplantation.[3] These events induce inflammation and oxidative stress favoring the release of reactive oxygen species, inflammatory mediators such as adhesion molecules, chemokines, cytokines, and endogenous ligands.[2] The resulting immune effects may enhance/activate a strong effector response that can lead eventually to graft rejection.[2] Indeed, many studies conducted on murine models of allograft rejection as well as some clinical trials have shown that blocking innate immunity activation led to a significant reduction in the rate of acute and chronic rejection.[2] Interestingly, the activation of the innate immune system that precedes the transplantation process is associated with the release of soluble mediators such as cytokines, miRNAs, eATP, PTX3, and sRAGE.[4] Here, we provide an overview on these novel soluble mediators and their role as contributors to the activation of the innate immune response in the context of solid allograft rejection.

CYTOKINES

Cytokines represent an important subset of soluble mediators of innate immunity released during/after solid allotransplantation involved in the development of the inflammatory response. The complexity and redundancy of the cytokine cascades activated during the alloimmune response make it difficult to fully dissect their relative functions. During solid organ transplantation, cytokines are usually released as a result of tissue damages, which is

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able to activate cellular components of the innate immune system in the early posttransplant phase and may therefore induce an inflammatory response.[5-7] Dendritic cells, mast cells, natural killer cells, macrophages, and other specialized cells of the immune system are responsible for the production of cytokines.[8] The release of proinflammatory cytokines, such as interleukin (IL)-6, IL-17, tumor necrosis factor alpha (TNF-α), IL-1β, granulocyte-macrophage colony-stimulating factor, interferon-γ, and monocyte chemotactic protein-1, is predominantly associated with the rapid influx of neutrophils, macrophages infiltration, and subsequent activation of mononuclear cell subsets within the graft.[9,10] The migration and infiltration of neutrophils to the site of acute inflammation following an increase in specific cytokines such as IL-8 and IL-17A occur rapidly (within min to h) with subsequent recruitment of additional cells of the immune system.[11,12] Once at the graft site, they undergo degranulation with the production of highly reactive oxygen metabolites contributing to graft rejection in solid organ transplantation.[11,13] Earlier studies conducted in toll-like receptor 4–deficient mice that received syngeneic heart transplantation showed lower rate of graft infiltration, reduced neutrophil infiltration as well as a decrease in the serum levels of proinflammatory cytokines including TNF-α, IL-6, monocyte chemotactic protein-1, and IL-1 (Table 1).[14] Other seminal studies have reported that in response to proinflammatory cytokines such as IL-1 and IL-6, macrophages are also recruited to the graft site, where they release degradative enzymes and reactive oxygen species contributing thus to acute and chronic allograft rejection.[15,16] Natural killer cells activated by IL-15 have been described to reject major histocompatibility complex mismatched allografts directly, without the involvement of other cell subsets of the adaptive immune system.[17] Proinflammatory cytokines such as TNF-α play a key role in early inflammatory responses after transplantation, TNF-α inhibition has been shown to decrease histological evidence of inflammation by reducing neutrophil migration into cardiac allografts.[18] In the initial stages of transplantation, IL-12 and IL-23 produced by antigen-presenting cells facilitate the differentiation of alloreactive T cells[19] and IL-12 and IL-23 antagonists prolonged cardiac allograft survival in mice.[20] Inflammatory cytokines such as IL-1α and IL-1β play a determinant role in mediating acute inflammation after transplantation.[21] The proof of concept of targeting the aforementioned cytokines has been established in autoimmune diseases. For instances, IL-12/IL-23 inhibitors ustekinumab and briakinumab have been studied in patients with psoriasis, psoriatic arthritis, and Crohn's disease, with cellulitis being the most reported serious infection.[22] Several studies showed a beneficial effect of IL-1β blockade by canakinumab, which led to a reduction in neutrophilic inflammation in the graft.[23,24] IL-17A antagonists such as secukinumab or ixekizumab may prevent allorecognition of the donor allograft and the mounting of immune response against the allograft.[25] A major target for therapeutic intervention is IL-6 that has led to development of several strategies that target either the cytokine directly, anti-IL-6 mAb (eg, clazakizumab) or its receptor, anti-IL6R mAb (eg, tocilizumab).[26] Recently, a phase 2 randomized pilot trial on kidney transplant recipients was used to evaluate the safety and efficacy of clazakizumab in late antibody-mediated rejection. Clazakizumab treatment was associated with decreased donor-specific antibodies and with reduction in the expression of rejection-related gene.[27] In a single-center, observational study, tocilizumab was used for treatment of acute antibody-mediated rejection in kidney-transplanted patients, further studies are needed to better define the benefit of IL-6 targeting.[28,29] Hence, clinical trials in the context of transplantation are needed to confirm the possible outcomes, whereas the appearance of some related-collateral effects warrants further attention for their potential safety and efficacy[3,24] (Table 2). The studies ascribed here indicate that cytokines are important contributors to solid allograft rejection and represent a relevant tool for the diagnosis of clinical rejection episodes, although some controversy remains. Particularly, for a cytokine-based immunosuppressive strategy to be successful, likely multiple cytokines should be targeted simultaneously, and this may limit this approach.

miRNA

Micro RNAs (miRNAs) represent an additional class of molecules that participate in connection with the innate immune activation during the inflammatory process following transplantation, leading to early allograft rejection. miRNAs described as short noncoding, single-stranded RNAs, have the ability to regulate critical cellular processes at the posttranscriptional level by inhibiting translation and inducing degradation of its target mRNA, depending on the degree of complementarity and accessibility of the binding sites.[30] Emerging data support the findings that miRNAs can also be stably found in a variety of body fluids, such as sera, saliva, urine, and blood, either packaged within exosomes, extracellular vesicles, or in complexes with RNA-binding proteins.[31] The presence of miRNAs in sera has been demonstrated as valuable biomarkers indicating heart allograft rejection and heart failure. In a recent clinical study (NCT02672683) reported by Xavier et al, the level of miR-10a, miR-155, miR-31, and miR-92a was found to be significantly different in the sera of patients with allograft rejection as compared to those without

TABLE 1.

Preclinical studies on soluble mediators

Target	Intervention	Effect	References
Cytokines	TLR4-deficiency	Lower rate of graft infiltration	Kaczorowski et al,[14] 2007
Cytokines	TNF-α antagonists	Reduction in neutrophilic migration into allograft	Ishii et al,[18] 2010
Cytokines	IL-12 and IL-23 antagonists	Prolong allograft survival	Wang et al,[20] 2012
miRNA	miR-155 antagomir	Attenuate rejection	Van Aelst et al,[41] 2016

IL, interleukin; miRNA, micro RNA; TLR, toll-like receptor; TNF, tumor necrosis factor.

TABLE 2.

Clinical trials on soluble mediators: cytokines

Title	Mediator	Condition or disease	NCT no.	Status	Study type	Phase
Cytokine profile postheart transplant	Cytokines	Cardiac transplant	NCT01643564	Terminated (no participants)	Observational	NA
Early indicators of chronic rejection in lung transplant	Cytokines	Chronic rejection in lung transplant, cytokine production in BOS post–lung transplant	NCT00449332	Terminated (no results posted)	Observational	NA
Cytokines evaluation in early calcineurin inhibitors withdrawn on renal transplant	Cytokines	Renal transplant rejection, immunosuppression	NCT01239472	Completed	Interventional	IV
Cytokine kinetics test to assess the presence or absence of tolerance in organ transplant	Cytokines	Kidney transplantation, liver transplantation	NCT00585858	Terminated (no results posted)	Observational	NA
Intraoperative use of extracorporeal cytokine adsorption during orthotopic heart transplantation	Cytokines	Cardiac transplantation, cardiopulmonary bypass	NCT03145441	Recruiting	Interventional	NA
HOPE with cytokine filtration in liver transplantation (cyto HOPE)	Cytokines	Liver transplantation, postreperfusion syndrome, ischemia-reperfusion injury, early allograft dysfunction	NCT04203004	Not yet recruiting	Interventional	NA
Effects of the use of "de novo" everolimus in renal transplant population	Cytokines	Delayed function of renal transplant	NCT01663805	NA	Interventional	IV
Acute renal failure postliver transplantation	Cytokines	Acute renal failure	NCT01907061	Completed	Interventional	NA
Effect of transversus abdominis plane block on anti-inflammatory response	Cytokines	Liver transplant; complications, inflammatory response	NCT04232904	Recruiting	Interventional	IV
EPI-STORM: cytokine storm in organ donors	Cytokines	Organ donation, liver transplantation, kidney transplantation, graft dysfunction	NCT03786991	Recruiting	Observational	NA
Evolution of interleukin 7, fat mass, and metabolic profile before and after transplantation	Cytokines	Immunodeficiency secondary to organ transplantation	NCT01414660	Completed	Observational	NA

BOS, bronchiolitis obliterans syndrome; HOPE, hypothermic oxygenated perfusion; NA, not applicable.

TABLE 3.
Clinical trials on soluble mediators: miRNAs and eATP

Title	Mediator	Condition or disease	NCT no.	Status	Study type	Phase
miRNA in kidney transplantation: association with kidney graft function and disease process	miRNA	Kidney transplant failure and rejection	NCT04413916	Completed	Observational	NA
Noninvasive detection of cardiac allograft rejection by circulating microRNAs (MIRRACLE)	miRNA	Cardiac transplantation	NCT02672683	NA	Observational	NA
MicroRNA expression in everolimus-based vs tacrolimus-based regimens in kidney transplantation	miRNA	miRNA profiles	NCT02091973	NA	Interventional	NA
A P2X7R single nucleotide mutation promotes chronic allograft vasculopathy	eATP	Cardiac allograft vasculopathy	NCT02082821	NA	Observational	NA

eATP, extracellular ATP; miRNA, micro RNA; NA, not applicable.

rejection.[32] Importantly, the 4 aforementioned miRNAs (miR-10a, miR-155, miR-31, and miR-92a) were reported to be associated with inflammatory pathways, cardiomyocytes/interstitial cells, and endothelial cells.[32] It is not surprising to see that circulating miRNAs play a key role in the overall inflammatory response toward graft rejection, as an increasing number of studies have demonstrated that circulating miRNAs play a direct role in the immune system.[33] For example, circulating miR-21 is known to affect innate immune system by acting as ligands to toll-like receptor (TLR)7 and TLR8, thus promoting the release of TNF-α and IL-6 by macrophages.[16,34] Circulating miR-223 has been described to play a determinant role during vesicle-induced monocyte maturation and to be part of a feedback mechanism inducing the differentiation of recruited monocytes and an increased release of vesicles as a local response activating the innate immune system.[35] The crosstalk between dendritic cells has also been implied in different and specific biological roles, such as the increase in the release of miR-155 enhances the inflammatory response, whereas miR-146a reduces it by mediating target gene repression and reprogramming the response to endotoxins.[36] Hence, monitoring the circulating levels of miR-133a, miR-208a, miR-499, and miR-133b at different time points posttransplantation could provide valuable information in predicting graft dysfunction and damage.[37,38] A study by Feng et al, showed that circulating miR-133a, miR-142, miR-146a, miR-208a, miR-34a, and miR-122 induce an inflammatory response in innate immune cells and cardiac myocytes.[39] The authors reported that miR-133a and miR-146a induced neutrophil and monocyte recruitment and further identified a pivotal role for TLR7 and MyD88 signaling in mediating miRNA proinflammatory effects that led to the conclusion that miRNAs are potent TLR7 ligands contributing to the activation of an innate immune response.[39] Indeed, some of these miRNAs could not only be biomarkers but also therapeutic target, as they are able to induce an inflammatory response and therefore activate innate immune cells.[39] miRNAs might represent potent TLR7 ligands contributing to the activation of an innate immune response,[39] and it is well known that TLRs and MYD88 are instrumental in the development of acute and chronic rejection.[40] Hence, miRNA-based therapeutic strategies could prove to be a viable option that might improve transplantation outcomes. One such study in which miRNA inhibitors with 2′-Omethyl modification have inhibited TLR7/8 these have the capacity to exhibit dual activities on inflammation through steric miRNA sequestration and TLR7/8 inhibition.[34] Other groups have already applied such methods in which an antagomir, a synthetically derived oligonucleotide sequence that complementary binds to the miRNA, was able to suppress miR-155 activity and attenuate allograft rejection in mice[41] as well as miR-208a was demonstrated to have an effect in abrogating other conditions[42] (Table 1). Recently, our group demonstrated that miR-21 is the most highly expressed miRNA in transplanted hearts with allograft vasculopathy and that targeting miR-21 delays chronic allograft vasculopathy onset by reprogramming macrophages metabolism.[16] However, the diagnostic and therapeutic potential of miRNAs as biomarkers in the clinical setting are limited, as methods used for the detection of miRNAs from serum, plasma, and urine samples need to be standardized. Furthermore, a

better insight is needed into mechanisms involved in post-transcriptional regulation of miRNAs as well as into the pathways responsible for the rejection process since their expression does not always correlate perfectly with that of host genes. Additionally, large-scale multicentered clinical studies are needed before they can be used in clinical practice. One of the many challenges of miRNA-based therapeutics is their unique chemistry, which makes them susceptible to degradation devoid of any selective uptake or any off-target effects. Many miRNAs that might be considered as therapeutic targets are also involved in other disease processes, such as oncogenesis. Nonetheless, significant advances have been made in the area[43]; nanoparticle delivery is possible and miRNA mimics and inhibitors can be used in combination with nanoparticles.[44] Several miRNA-based clinical trials are taking place, including those for hepatitis C, type II diabetes mellitus, and others, which warrants further attention[45] (Table 3). Taken together, these studies pave the way for the development of miRNA-based therapeutic strategies in transplantation aiming at suppressing innate immunity and at inducing immune tolerance.

Extracellular ATP

Similar to miRNAs, extracellular ATP (eATP) and subsequent purinergic signaling might to be considered as inflammatory mediator in solid organ transplantation acting as an extracellular signal promoting immune cell activation and infiltration. In fact, endogenous ATP (ATP) is highly released during allotransplantation and may act as a danger signal. ATP can be released as eATP during ischemia reperfusion or upon the activation of immune cells or by necrotic/apoptotic cells during the peritransplant period.[46] eATP is recognized by specific cell surface nucleoside receptors or the ionotropic P2X as well as by metabotropic P2Y purinergic receptors, particularly P2X7R.[47] During organ transplantation, eATP/P2X7R signaling has been evidently reported as an important interplayer during allograft rejection and the resulting alloimmune response.[48] Furthermore, ATP release might provoke additional graft damage, a process that self-supports the antigraft immune response.[46] eATP release into the circulation during cellular injury could activate innate immune receptors and signaling pathways and therefore promote inflammation as it might also possibly lead to host tissue damage.[49] Upon binding of extracellular ATP to P2X or P2Y receptors might trigger a wide range of immune and inflammatory effects on leukocytes. eATP signaling contributes to other fundamental functions such as cytokine production, phagocytic activity, and migration modulation by monocytes, neutrophils, eosinophils, dendritic cells, and macrophages.[50] In the context of solid organ transplantation, the direct relevance of eATP has been extensively addressed by our group in which P2X7R, found predominantly expressed on immune cells (eg, mast cells, macrophages, and dendritic cells), and its signaling through eATP has been described to affect the outcomes of allogenic transplantation.[51] Indeed, ATP secreted by damaged/ischemic grafts engages P2X7R on antigen-presenting cells, thereby creating a proinflammatory milieu that may, in turn, facilitate direct allorecognition, representing thus a barrier to tolerance in allotransplantation. Particularly, P2X7R inhibition or conditional deficiency on dendritic cells was found associated with reduced severity of graft-versus-host disease.[52] In fact, eATP-P2X7R signaling in antigen-presenting dendritic cells has led to an increased expression of some costimulatory molecules such as CD80 and CD86 in vitro and in vivo and activated a cascade of proinflammatory events.[52] Dendritic cells, macrophages, neutrophils, and eosinophils were also found also to express different P2Y receptors, which regulate cytokine production, antigen capture, cell maturation, and migration, thus promoting the inflammatory response and tissue damage.[46,53,54] P2YRs have been studied as a pharmacological target for the treatment of inflammation given their major role in the acceptance or rejection of the allograft.[55,56] In particular, the expression of P2Y1 and P2Y2R on immune cells is deemed important for immunomodulation and inflammation, which occur after organ transplantation.[53,57] Grafted tissues are affected by ischemia/reperfusion that promotes immune cell activation and infiltration. Growing evidences have revealed that targeting P2Y receptors such as P2Y2, P2Y4, and P2Y11 may be beneficial thereby conferring cardiac protection by reducing cardiac fibrosis and neointima formation.[58–60] Although the targeting of P2Y receptor signaling may be beneficial for graft rejection, several challenges need to be addressed, including the development of subtype-highly specific P2YR antagonists. Furthermore, understanding the involvement of the different components of the purinergic system in organ transplantation will pave the way for designing therapeutic strategies aimed at targeting the purinergic system and at blocking the eATP signaling in solid organ transplant (Table 3).

OTHER SOLUBLE MEDIATORS

The experimental and clinical studies described above underlined a crucial role for cytokines, miRNAs, and eATP in the activation of the innate immune system during solid organ transplantation. Moreover, other soluble mediators may be involved in the activation of the immune innate system. Pentraxin 3 (PTX3) and soluble receptor for advanced glycation end products (sRAGE) are multifunctional proteins with complex regulatory roles in inflammation and extracellular matrix organization and remodeling. They play key roles in initiating, maintaining, and resolving tissue inflammation, as well as in the functioning of the innate immune system, with a likely role in allograft injury and protection. PTX3 and sRAGE may be defined as novel soluble mediators for their potential use as diagnostic/prognostic factors since their plasma levels might reflect the extent of tissue damage and might predict the risk of mortality. Furthermore, PTX3 and sRAGE may represent potential targets for future therapeutic strategies aimed at the regulation of inflammation and at the reversal of vascular abnormalities in the setting of solid organ transplantation (Figure 1).

Pentraxin 3

Pentraxin 3 (PTX3) is produced at the sites of inflammation or injury and plays a major role in innate immunity.[61] PTX3 is a multifunctional soluble pattern recognition receptor identified as the more recently discovered long pentraxin.[62] PTX3 blood levels are low under

normal conditions but increase rapidly and dramatically in response to inflammatory stimuli.[63] PTX3 has been described to exert regulatory functions on innate immunity and during inflammation.[64] Preclinical studies using PTX3-transgenic mice showed exacerbated inflammatory response following ischemia-reperfusion injury associated with higher inflammatory response and lethality rates due to the enhanced production of proinflammatory mediators, including nitric oxide and TNF-α.[65] Some in vitro studies suggested that PTX3 might influence the expression of CD86 and MHC class I/II by human-derived macrophages and by dendritic cells or the upregulation of tissue factor expression in human monocytes upon its stimulation by lipopolysaccharides.[66-68] The function of PTX3 in solid organ transplantation has not been studied in details, but recent studies reported significant increase in plasma levels of PTX3 and changes in PTX3 expression in graft biopsies after lung and kidney transplantation. These changes in PTX3 were associated with worst outcomes and the development of primary graft dysfunction and acute renal allograft rejection.[69,70] Other studies have been carried out to further investigate the influence of PTX3 on macrophages, which seems to favor the release of TGF-β.[67] PTX3 might also inhibit phagocytosis of late apoptotic cells by macrophages and dendritic cells.[67,71] A study using a PTX3 KO mice in a murine model of lung transplantation underlined that PTX3 may be also protective in the long-term of chronic.[72] The ambiguous role of endogenous PTX3 warrants further attention and investigation before its potential translation.[73-75] PTX3 could be potentially involved in multiple physiological function and fully understanding its role may facilitate the development of targeted therapeutic approaches in solid organ transplantation.[65,76,77]

sRAGE

The receptor for advanced glycation end products (RAGE) is involved in several processes including tissue regeneration, inflammatory response, and modulation of alloimmune response. RAGEs can be expressed as a soluble form; sRAGE is likely to act as a decoy molecule that binds excess RAGE ligand.[78,79] Recent studies indicate that sRAGE possesses both anti-inflammatory and proinflammatory properties depending on the cellular composition and on the availability of ligands in the tissue environment.[78] The protective effect of sRAGE was demonstrated in a murine model of heart transplantation, which procured

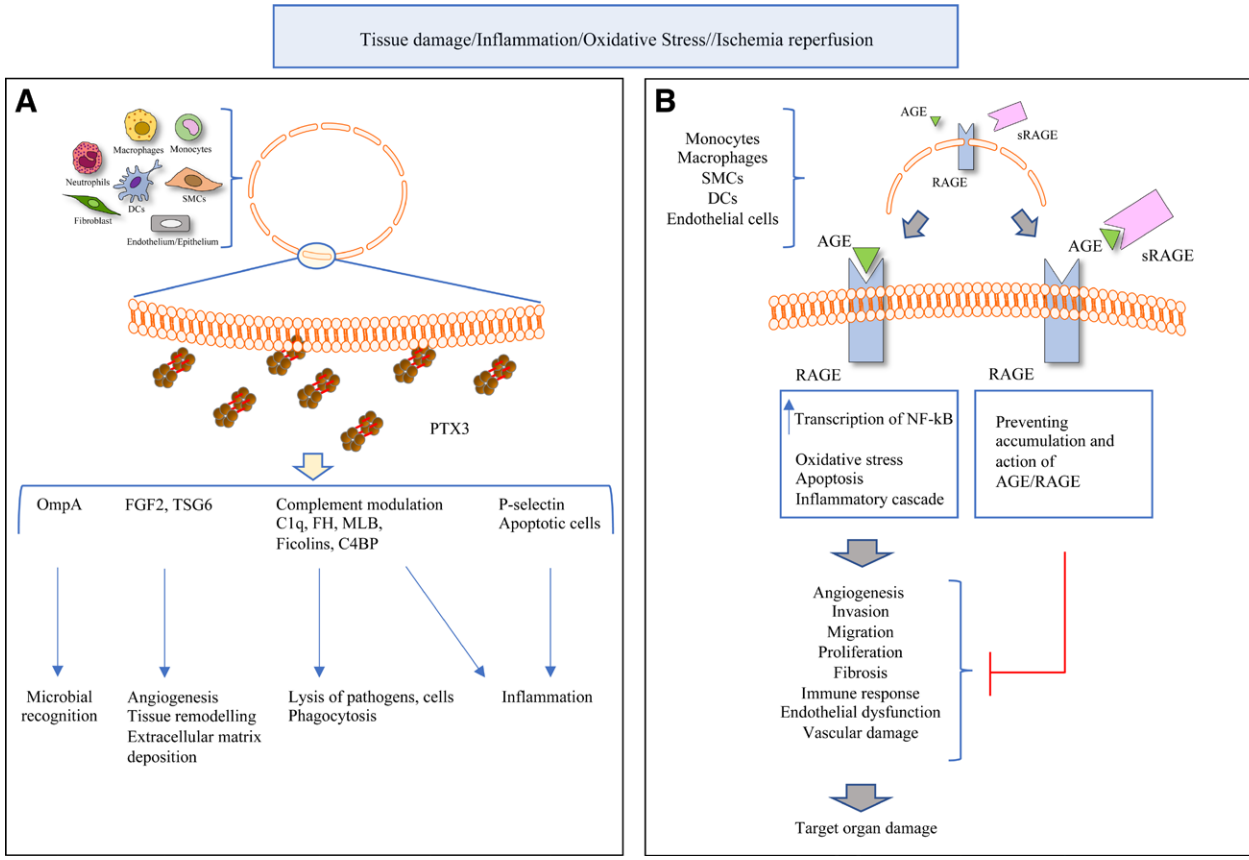

FIGURE 1. Schematic representation of the roles of PTX3 and sRAGE in innate immunity. A, Tissue damage, vascular inflammation, or ischemia reperfusion following transplantation induce PTX3 release by monocytes, neutrophils, macrophages, SMCs, endothelial cells, epithelial cells, DCs, and fibroblasts. This released PTX3 interacts with ligands playing an important role in the regulation of inflammation, tissue remodeling, and innate immunity. B, Inflammation, oxidative stress, ischemia reperfusion, and more general cell stress release AGE, which binds to RAGE on monocytes, macrophages, SMCs, DCs, endothelial cells, and activate signaling cascades that enhance immune response, endothelial dysfunction, and vascular damage lead to organ damage. AGE, advanced glycation end products; C1q, complement component 1q; C4BP, C4b binding protein; DC, dendritic cell; FGF2, fibroblast growth factor 2; FH, factor H; MLB, mannose-binding lectin; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; OmpA, outer membrane protein A; PTX3, pentraxin 3; RAGE, receptor for AGE; SMC, smooth muscle cell; sRAGE, soluble RAGE; TSG6, tumor necrosis factor α-stimulated gene-6.

a protection to the graft from I/R by attenuating the inflammatory response and reducing neutrophil infiltration.[80] In vitro studies demonstrated that sRAGE may bind directly to phagocytes and lead to the activation of Akt, Erk, and NF-κB signaling pathways, thus promoting their survival, migration, and even differentiation into macrophages.[78,80] Additional studies have reported that sRAGE treatment may induce human monocyte and neutrophil recruitment/migration[78] or prime them to release several proinflammatory cytokines and chemokines.[81] To date, only few associations between the levels of sRAGE and transplantation have been described. A single study described that after liver transplantation, the levels of circulating sRAGE decreased by day 7 posttransplantation suggesting thus that sRAGE might exert inhibitory effects on RAGE, where decreased levels of sRAGE may contribute

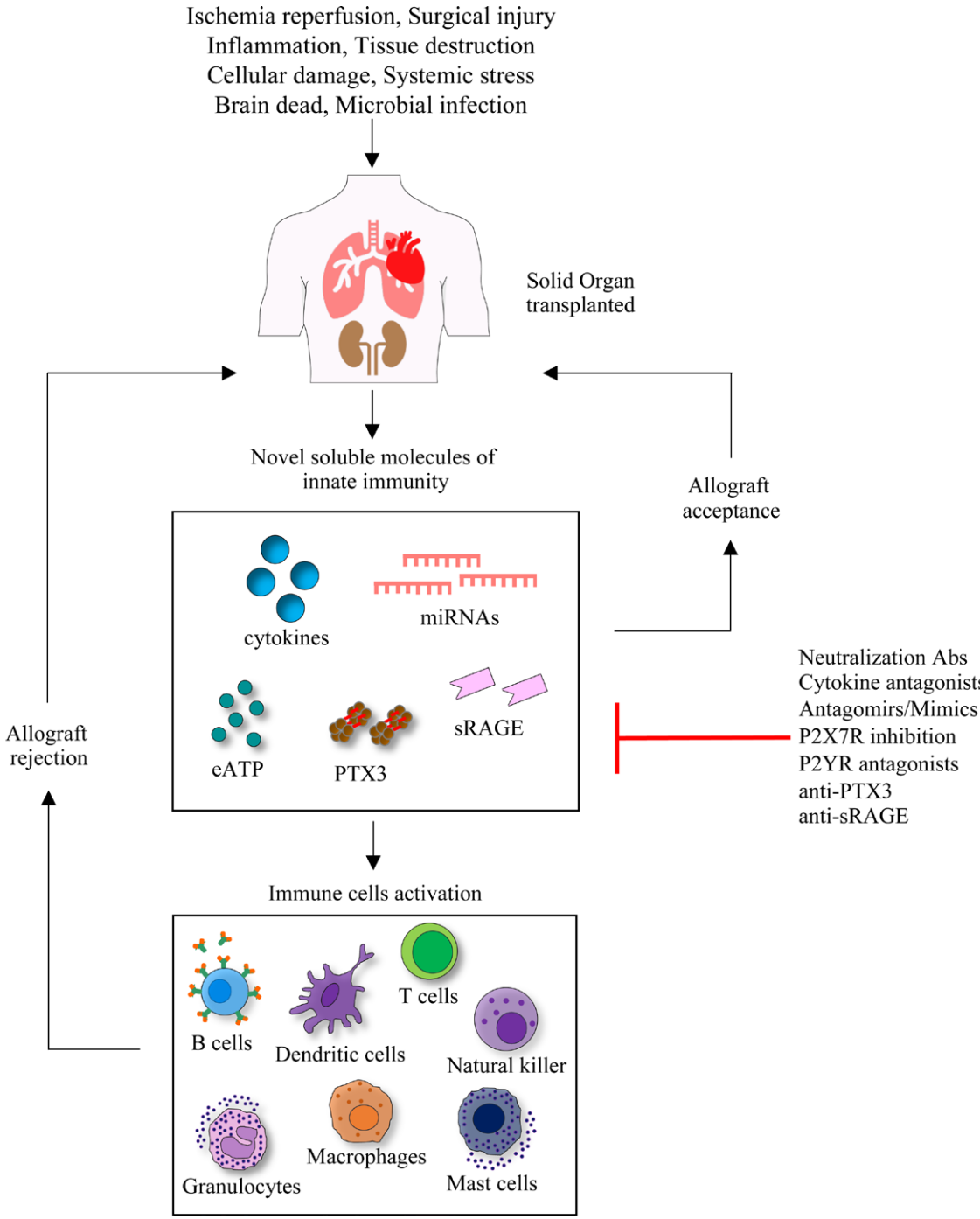

FIGURE 2. Novel soluble mediators of the innate immunity and therapeutic approaches for allograft rejection. A series of events, such as ischemia/reperfusion, surgical injury, inflammation, tissue destruction, cellular damage, systemic stress, brain death, and microbial infection, occurring after solid organ transplantation lead innate immune activation triggering a release of soluble mediators. The induction of cytokines, miRNAs, eATP, PTX3, and sRAGE augments the alloreactive cells activation leading to cardiac allograft rejection. Evolving data suggest that using therapeutic strategy to block the activation of the soluble mediators could avoid potentially organ fibrosis and chronic graft dysfunction. eATP, extracellular ATP; miRNA, microRNA; PTX3, pentraxin 3; P2X7R, P2X receptor 7; P2YR, P2Y receptor; sRAGE, soluble receptor for advanced glycation end products.

to enhanced RAGE-mediated proinflammatory signaling after transplantation and during I/R injury.[82] Moreover, elevated plasmatic levels of sRAGE in transplant recipient and mainly 24 h after reperfusion were associated with the development of lung primary graft dysfunction within the first 72 h after transplant.[83] Hence, improving our understanding of the precise function of sRAGE will likely provide better therapeutic approaches for the treatment of RAGE-related events.[78] In fact, further studies are needed to link the levels of sRAGE to the status or severity of the disease. No available evidence is solid enough to conclude that sRAGE levels are associated with disease risk. It is predictable that sRAGE will be used more as a therapeutic target rather than just a biomarker, but long-term prospective clinical studies are required to test this hypothesis. Moreover, whether there might emerge some side effects after long-term treatment with RAGE or soluble RAGE antagonists and whether these approaches exert deleterious effects remains to be fully determined.[84] Blockade of RAGE signaling itself represents the most reasonable approach to be pursued as therapeutics.[85] Given their involvement in the regulation of the immune system, however, circulating sRAGE deserves a place in future research, mainly in the context of solid organ transplantation.[86]

CONCLUSION

Available clinical and experimental data established that multiple events including brain donor death, ischemia-reperfusion injury, surgical injury, inflammation, tissue/cell damage, and infection have led to the activation of innate immune response and in turn direct adaptive immunity response leading to solid allograft rejection. Particularly the activation of the innate immune system in the early phase after solid organ transplantation is mainly a nonspecific response to tissue damage. This review supports the view that this process is mediated via signaling through secretion of various soluble factors such as the cytokines, miRNAs, eATP, PTX3, and sRAGE by killing the donor cells or by promoting and amplifying deleterious inflammatory adaptive immune responses. Production of these soluble factors activates a cascade that generates a variety of effector molecules that can harm the graft, favor antigen presentation, and combine the innate and adaptive immune responses. For some types of transplant, it is reasonable that donor tissue may produce and release soluble factors locally in the graft, thus potentially amplifying the early response to the transplant. Improving our understanding of how soluble factors are involved in the activation of innate immune system may be very relevant to the search of new therapeutic strategies employing agents designed to suppress such factors to mitigate solid allograft rejection. Hence, strategies based on blocking the deleterious signaling of these soluble factors will likely improve allograft outcomes and allow for the minimization of systemic immunosuppressive therapies; hence, controlling the innate response might be an effective method of preventing solid allograft rejection (Figure 2).

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REFERENCES

1. Yang B, Jain S, Pawluczyk IZA, et al. Inflammation and caspase activation in long-term renal ischemia/reperfusion injury and immunosuppression in rats. *Kidney Int*. 2005;68:2050–2067.
2. Land WG. Injury to allografts: innate immune pathways to acute and chronic rejection. *Saudi J Kidney Dis Transpl*. 2005;16:520–539.
3. Benichou G, Tonsho M, Tocco G, et al. Innate immunity and resistance to tolerogenesis in allotransplantation. *Front Immunol*. 2012;3:73.
4. Solini A, Usuelli V, Fiorina P. The dark side of extracellular ATP in kidney diseases. *J Am Soc Nephrol*. 2015;26:1007–1016.
5. Land WG. The role of postischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. *Transplantation*. 2005;79:505–514.
6. Mueller FB, Yang H, Lubetzky M, et al. Landscape of innate immune system transcriptome and acute T cell-mediated rejection of human kidney allografts. *JCI Insight*. 2019;4:e128014.
7. Ochando J, Fayad ZA, Madsen JC, et al. Trained immunity in organ transplantation. *Am J Transplant*. 2020;20:10–18.
8. Lacy P, Stow JL. Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. *Blood*. 2011;118:9–18.
9. de Vries DK, Lindeman JH, Ringers J, et al. Donor brain death predisposes human kidney grafts to a proinflammatory reaction after transplantation. *Am J Transplant*. 2011;11:1064–1070.
10. Liang EC, Rossetti M, Sidwell T, et al. Differences in proinflammatory cytokines and monocyte subtypes in older as compared with younger kidney transplant recipients. *Transplant Direct*. 2018;4:e348.
11. Zhu H, Li J, Wang S, et al. Hmgb1-TLR4-IL-23-IL-17A axis promote ischemia-reperfusion injury in a cardiac transplantation model. *Transplantation*. 2013;95:1448–1454.
12. Mayer-Barber KD, Yan B. Clash of the cytokine titans: counter-regulation of interleukin-1 and type I interferon-mediated inflammatory responses. *Cell Mol Immunol*. 2017;14:22–35.
13. Bassi R, Niewczas MA, Biancone L, et al. Metabolomic profiling in individuals with a failing kidney allograft. *PLoS One*. 2017;12:e0169077.
14. Kaczorowski DJ, Nakao A, Mollen KP, et al. Toll-like receptor 4 mediates the early inflammatory response after cold ischemia/reperfusion. *Transplantation*. 2007;84:1279–1287.
15. Wood KJ, Goto R. Mechanisms of rejection: current perspectives. *Transplantation*. 2012;93:1–10.
16. Usuelli V, Ben Nasr M, D'Addio F, et al. miR-21 antagonism reprograms macrophage metabolism and abrogates chronic allograft vasculopathy. *Am J Transplant*. 2021;21:3280–3295.
17. Millington TM, Madsen JC. Innate immunity and cardiac allograft rejection. *Kidney Int Suppl*. 2010;119:S18–S21.
18. Ishii D, Schenk AD, Baba S, et al. Role of TNFalpha in early chemokine production and leukocyte infiltration into heart allografts. *Am J Transplant*. 2010;10:59–68.
19. Chan IH, Jain R, Tessmer MS, et al. Interleukin-23 is sufficient to induce rapid de novo gut tumorigenesis, independent of carcinogens, through activation of innate lymphoid cells. *Mucosal Immunol*. 2014;7:842–856.
20. Wang S, Xu X, Xie A, et al. Anti-interleukin-12/23p40 antibody attenuates chronic rejection of cardiac allografts partly via inhibition γδT cells. *Clin Exp Immunol*. 2012;169:320–329.
21. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2018;281:8–27.
22. Szczepanik A, Iasella CJ, McDyer JF, et al. Cytokine-targeted therapy for the management of solid organ transplant recipients. *Hum Immunol*. 2019;80:184–190.
23. Wanderer AA. Rationale and timeliness for IL-1beta-targeted therapy to reduce allogeneic organ injury at procurement and to diminish risk of rejection after transplantation. *Clin Transplant*. 2010;24:307–311.
24. van den Bosch TPP, Kannegieter NM, Hesselink DA, et al. Targeting the monocyte-macrophage lineage in solid organ transplantation. *Front Immunol*. 2017;8:153.
25. Patel DD, Lee DM, Kolbinger F, et al. Effect of IL-17A blockade with secukinumab in autoimmune diseases. *Ann Rheum Dis*. 2013;72(Suppl 2):ii116–ii123.
26. Jones SA, Fraser DJ, Fielding CA, et al. Interleukin-6 in renal disease and therapy. *Nephrol Dial Transplant*. 2015;30:564–574.
27. Doberer K, Duerr M, Halloran PF, et al. A randomized clinical trial of anti-IL-6 antibody clazakizumab in late antibody-mediated kidney transplant rejection. *J Am Soc Nephrol*. 2021;32:708–722.

28. Pottebaum AA, Venkatachalam K, Liu C, et al. Efficacy and safety of tocilizumab in the treatment of acute active antibody-mediated rejection in kidney transplant recipients. *Transplant Direct*. 2020;6:e543.
29. Choi J, Aubert O, Vo A, et al. Assessment of tocilizumab (anti-interleukin-6 receptor monoclonal) as a potential treatment for chronic antibody-mediated rejection and transplant glomerulopathy in HLA-sensitized renal allograft recipients. *Am J Transplant*. 2017;17:2381–2389.
30. Zhou SS, Jin JP, Wang JQ, et al. miRNAS in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges. *Acta Pharmacol Sin*. 2018;39:1073–1084.
31. Min PK, Chan SY. The biology of circulating microRNAs in cardiovascular disease. *Eur J Clin Invest*. 2015;45:860–874.
32. Duong Van Huyen JP, Tible M, Gay A, et al. MicroRNAs as non-invasive biomarkers of heart transplant rejection. *Eur Heart J*. 2014;35:3194–3202.
33. de Candia P, De Rosa V, Casiraghi M, et al. Extracellular RNAs: a secret arm of immune system regulation. *J Biol Chem*. 2016;291:7221–7228.
34. Sarvestani ST, Stunden HJ, Behlke MA, et al. Sequence-dependent off-target inhibition of TLR7/8 sensing by synthetic microRNA inhibitors. *Nucleic Acids Res*. 2015;43:1177–1188.
35. Ismail N, Wang Y, Dakhlallah D, et al. Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. *Blood*. 2013;121:984–995.
36. Alexander M, Hu R, Runtsch MC, et al. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. *Nat Commun*. 2015;6:7321.
37. Wang E, Nie Y, Zhao Q, et al. Circulating miRNAs reflect early myocardial injury and recovery after heart transplantation. *J Cardiothorac Surg*. 2013;8:165.
38. De Rosa S, Fichtlscherer S, Lehmann R, et al. Transcoronary concentration gradients of circulating microRNAs. *Circulation*. 2011;124:1936–1944.
39. Feng Y, Zou L, Yan D, et al. Extracellular MicroRNAs induce potent innate immune responses via TLR7/MyD88-dependent mechanisms. *J Immunol*. 2017;199:2106–2117.
40. Braza F, Brouard S, Chadban S, et al. Role of TLRs and DAMPs in allograft inflammation and transplant outcomes. *Nat Rev Nephrol*. 2016;12:281–290.
41. Van Aelst LN, Summer G, Li S, et al. RNA profiling in human and murine transplanted hearts: identification and validation of therapeutic targets for acute cardiac and renal allograft rejection. *Am J Transplant*. 2016;16:99–110.
42. Montgomery RL, Hullinger TG, Semus HM, et al. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. *Circulation*. 2011;124:1537–1547.
43. Khvorova A, Watts JK. The chemical evolution of oligonucleotide therapies of clinical utility. *Nat Biotechnol*. 2017;35:238–248.
44. Chen Y, Gao DY, Huang L. In vivo delivery of miRNAs for cancer therapy: challenges and strategies. *Adv Drug Deliv Rev*. 2015;81:128–141.
45. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*. 2017;16:203–222.
46. Vergani A, Tezza S, Fotino C, et al. The purinergic system in allotransplantation. *Am J Transplant*. 2014;14:507–514.
47. Junger WG. Immune cell regulation by autocrine purinergic signalling. *Nat Rev Immunol*. 2011;11:201–212.
48. D'Addio F, Vergani A, Potena L, et al. P2X7R mutation disrupts the NLRP3-mediated Th program and predicts poor cardiac allograft outcomes. *J Clin Invest*. 2018;128:3490–3503.
49. Iyer SS, Pulskens WP, Sadler JJ, et al. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc Natl Acad Sci U S A*. 2009;106:20388–20393.
50. Myrtek D, Idzko M. Chemotactic activity of extracellular nucleotides on human immune cells. *Purinergic Signal*. 2007;3:5–11.
51. Vergani A, Tezza S, D'Addio F, et al. Long-term heart transplant survival by targeting the ionotropic purinergic receptor P2X7. *Circulation*. 2013;127:463–475.
52. Wilhelm K, Ganesan J, Müller T, et al. Graft-versus-host disease is enhanced by extracellular ATP activating P2X7R. *Nat Med*. 2010;16:1434–1438.
53. Di Virgilio F, Vuerich M. Purinergic signaling in the immune system. *Auton Neurosci*. 2015;191:117–123.
54. Cekic C, Linden J. Purinergic regulation of the immune system. *Nat Rev Immunol*. 2016;16:177–192.
55. Dormond O, Dufour M, Seto T, et al. Targeting the intragraft microenvironment and the development of chronic allograft rejection. *Hum Immunol*. 2012;73:1261–1268.
56. Antonioli L, Blandizzi C, Pacher P, et al. The purinergic system as a pharmacological target for the treatment of immune-mediated inflammatory diseases. *Pharmacol Rev*. 2019;71:345–382.
57. Burnstock G. Purinergic signalling: therapeutic developments. *Front Pharmacol*. 2017;8:661.
58. Benoist L, Chadet S, Genet T, et al. Stimulation of P2Y11 receptor protects human cardiomyocytes against hypoxia/reoxygenation injury and involves PKCε signaling pathway. *Sci Rep*. 2019;9:11613.
59. Hochhauser E, Cohen R, Waldman M, et al. P2Y2 receptor agonist with enhanced stability protects the heart from ischemic damage in vitro and in vivo. *Purinergic Signal*. 2013;9:633–642.
60. Horckmans M, Esfahani H, Beauloye C, et al. Loss of mouse P2Y4 nucleotide receptor protects against myocardial infarction through endothelin-1 downregulation. *J Immunol*. 2015;194:1874–1881.
61. Kabbani D, Bhaskaran A, Singer LG, et al. Pentraxin 3 levels in bronchoalveolar lavage fluid of lung transplant recipients with invasive aspergillosis. *J Heart Lung Transplant*. 2017;36:973–979.
62. Garlanda C, Bottazzi B, Bastone A, et al. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol*. 2005;23:337–366.
63. Muller B, Peri G, Doni A, et al. Circulating levels of the long pentraxin PTX3 correlate with severity of infection in critically ill patients. *Crit Care Med*. 2001;29:1404–1407.
64. Bottazzi B, Garlanda C, Cotena A, et al. The long pentraxin PTX3 as a prototypic humoral pattern recognition receptor: interplay with cellular innate immunity. *Immunol Rev*. 2009;227:9–18.
65. Souza DG, Soares AC, Pinho V, et al. Increased mortality and inflammation in tumor necrosis factor-stimulated gene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol*. 2002;160:1755–1765.
66. Baruah P, Propato A, Dumitriu IE, et al. The pattern recognition receptor PTX3 is recruited at the synapse between dying and dendritic cells, and edits the cross-presentation of self, viral, and tumor antigens. *Blood*. 2006;107:151–158.
67. Shiraki A, Kotooka N, Komoda H, et al. Pentraxin-3 regulates the inflammatory activity of macrophages. *Biochem Biophys Rep*. 2016;5:290–295.
68. Napoleone E, di Santo A, Peri G, et al. The long pentraxin PTX3 upregulates tissue factor in activated monocytes: another link between inflammation and clotting activation. *J Leukoc Biol*. 2004;76:203–209.
69. Imai N, Nishi S, Yoshita K, et al. Pentraxin-3 expression in acute renal allograft rejection. *Clin Transplant*. 2012;26(Suppl 24):25–31.
70. Diamond JM, Lederer DJ, Kawut SM, et al; Lung Transplant Outcomes Group. Elevated plasma long pentraxin-3 levels and primary graft dysfunction after lung transplantation for idiopathic pulmonary fibrosis. *Am J Transplant*. 2011;11:2517–2522.
71. van Rossum AP, Fazzini F, Limburg PC, et al. The prototypic tissue pentraxin PTX3, in contrast to the short pentraxin serum amyloid P, inhibits phagocytosis of late apoptotic neutrophils by macrophages. *Arthritis Rheum*. 2004;50:2667–2674.
72. Yoshida M, Oishi H, Martinu T, et al. Pentraxin 3 deficiency enhances features of chronic rejection in a mouse orthotopic lung transplantation model. *Oncotarget*. 2018;9:8489–8501.
73. Magrini E, Mantovani A, Garlanda C. The dual complexity of PTX3 in health and disease: a balancing act? *Trends Mol Med*. 2016;22:497–510.
74. Brilland B, Vinatier E, Subra JF, et al. Anti-pentraxin antibodies in autoimmune diseases: bystanders or pathophysiological actors? *Front Immunol*. 2020;11:626343.
75. de Oliveira THC, Souza DG, Teixeira MM, et al. Tissue dependent role of PTX3 during ischemia-reperfusion injury. *Front Immunol*. 2019;10:1461.
76. Souza DG, Amaral FA, Fagundes CT, et al. The long pentraxin PTX3 is crucial for tissue inflammation after intestinal ischemia and reperfusion in mice. *Am J Pathol*. 2009;174:1309–1318.
77. Lech M, Römmele C, Gröbmayr R, et al. Endogenous and exogenous pentraxin-3 limits postischemic acute and chronic kidney injury. *Kidney Int*. 2013;83:647–661.
78. Wang Y, Wang H, Piper MG, et al. sRAGE induces human monocyte survival and differentiation. *J Immunol*. 2010;185:1822–1835.
79. Ramasamy R, Schmidt AM. Receptor for advanced glycation end products (RAGE) and implications for the pathophysiology of heart failure. *Curr Heart Fail Rep*. 2012;9:107–116.
80. Zhang X, Cao X, Dang M, et al. Soluble receptor for advanced glycation end-products enhanced the production of IFN-γ through the

NF-κB pathway in macrophages recruited by ischemia/reperfusion. *Int J Mol Med*. 2019;43:2507–2515.
81. Pullerits R, Brisslert M, Jonsson IM, et al. Soluble receptor for advanced glycation end products triggers a proinflammatory cytokine cascade via beta2 integrin Mac-1. *Arthritis Rheum*. 2006;54:3898–3907.
82. Navarra T, De Simone P, Del Turco S, et al. Involvement of the receptor for advanced glycation end products in liver transplantation. *Ann Hepatol*. 2015;14:190–197.
83. Christie JD, Shah CV, Kawut SM, et al; Lung Transplant Outcomes Group. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. *Am J Respir Crit Care Med*. 2009;180:1010–1015.
84. Yan SF, Ramasamy R, Schmidt AM. Soluble RAGE: therapy and biomarker in unraveling the RAGE axis in chronic disease and aging. *Biochem Pharmacol*. 2010;79:1379–1386.
85. Kierdorf K, Fritz G. RAGE regulation and signaling in inflammation and beyond. *J Leukoc Biol*. 2013;94:55–68.
86. Pilzweger C, Holdenrieder S. Circulating HMGB1 and RAGE as clinical biomarkers in malignant and autoimmune diseases. *Diagnostics (Basel)*. 2015;5:219–253.