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Innate Immune Mechanisms in Transplant Allograft Vasculopathy

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Abstract

Purpose of Review—Allograft vasculopathy (AV) is the leading cause of late allograft loss following solid organ transplantation. Ischemia reperfusion injury (IRI) and donor specific antibody (DSA)-induced complement activation confer heightened risk for AV via numerous innate immune mechanisms including MyD88, HMGB1, and complement induced non-canonical NF- κ B signaling.

Recent Findings—The role of MyD88, a signal adaptor downstream of the toll-like receptors (TLR), has been defined in an experimental heart transplant model, which demonstrated that recipient MyD88 enhanced AV. Importantly, triggering receptor on myeloid receptor 1 (Trem1), a MyD88 amplifying signal, was present in rejecting human cardiac transplant biopsies and enhanced the development of AV in mice. HMGB1, a nuclear protein that activates TLRs, also enhanced the development of AV. Complement activation elicits assembly of membrane attack complexes (MAC) on endothelial cells which activate non-canonical NF- κ B signaling, a novel complement effector pathway that induces pro-inflammatory genes and potentiates endothelial cell mediated alloimmune T cell activation, processes which enhance AV.

Summary—Innate immune mediators including HMGB1, MyD88, and non-canonical NF κ B signaling via complement activation contribute to AV. These pathways represent potential therapeutic targets to reduce AV after solid organ transplantation.

Keywords

toll-like receptor; MyD88; non-canonical NF- κ B; membrane attack complex; allograft vasculopathy

INTRODUCTION

Allograft vasculopathy (AV) is a highly prevalent vaso-occlusive condition that affects up to 51% of patients 10 years post-transplant [1]. In AV, neointimal and less commonly thrombotic lesions form in large- and small-caliber vessels of the transplanted graft, leading to ischemic complications and allograft loss. In the setting of heart transplantation, where

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AV is most widely studied and is referred to as cardiac allograft vasculopathy or CAV, AV lesions affect the epicardial coronary and intramyocardial microvessels [2] and is recognized as the leading cause of graft loss after one year post-heart transplantation. Ischemia reperfusion injury (IRI) and development of donor specific antibodies (DSA) are both strong, independent risk factors for the development of AV.

AV is a fundamentally distinct entity from coronary artery disease (CAD). Given its unique pathophysiological underpinnings involving alloimmune T cell activation, AV is clinically refractory to many repositioned agents intended for treating CAD [3]. Moreover, contemporary immunosuppressive regimens to halt alloimmune adaptive responses have yielded only incremental or no therapeutic benefit regarding secondary or tertiary prevention [4,5]. Future agents specifically designed for treating AV are required, and for the rational design of such therapies an improved understanding of the mechanisms underlying AV is urgently needed. Here, we review the role of innate immune signaling molecules and pathways, including HMGB, MyD88 and complement induced non-canonical NF- κ B, in the development of AV.

MyD88: a central pathway enhancing chronic allograft rejection

MyD88 is a signal adaptor protein that is downstream of all the TLRs, except TLR3 and also IL-1 and IL-18. TLRs are key innate immune receptors that are expressed on stromal and hematopoietic cells and activated by both microbial and non-microbial activators [6,7]. MyD88 has been established to enhance acute allograft rejection and break transplant tolerance [8–10]. In humans, patients with low expression of MyD88 in peripheral blood mononuclear cells exhibit a significant increase in operational transplant tolerance as compared to renal transplant recipients with evidence of chronic rejection [11]. Mechanistically, MyD88 signaling in an experimental model of kidney transplantation abrogates spontaneous transplant tolerance [12]. MyD88 impaired the expansion of regulatory T cells (T_{regs}) and increased anti-donor IL-17 immune responses [12,13]. Interestingly, the authors of this study recently reported that IL-17 enhanced both acute and chronic allograft rejection in a spontaneous model of murine renal allograft acceptance [14]. The MyD88 expressing cells that mediate AV have not been determined. Prior studies have indicated that NK cells and macrophages may promote AV and represent potential possibilities [15, 16].

In a MHC class II mismatched murine cardiac allograft model, deficiency of MyD88 in the recipient enhanced cardiac allograft survival with reduced evidence of AV [17]. This study also found that there were increased numbers of Trem-1+ immune cells within human heart transplant biopsy specimens with evidence of acute graft rejection [17]. Trem1 is an immunoglobulin superfamily member that is expressed on a variety of innate immune cells including dendritic cells (DCs), macrophages and neutrophils acts as an amplifying signal for MyD88 [18]. Importantly, pharmacological inhibition of Trem1 enhanced cardiac allograft survival with reduced AV development in the murine MHC class II mismatched model [17]. Trem1 inhibition also reduced T cell allograft infiltration and reduced anti-donor IFN- γ T cell responses within the spleen of cardiac allograft recipients [17,18]. Thus, this study defines a role for Trem-1 as MyD88 amplification signal that enhances AV.

IRI leads to cellular necrosis and induces sterile inflammation [19]. During cellular necrosis, substances are released that are typically not sensed by the innate immune system [18,19]. High mobility group box 1 (HMGB1) is a nuclear protein that enhances gene transcription [20]. It has been shown to activate TLR 4 and TLR9, TLRs upstream of MyD88 [21–22]. Prior studies have found a role for HMGB1 in acute allograft rejection as well as cardiac, renal and hepatic IRI [23–27]. Recently, in a MHC class II mismatched cardiac allograft model, pharmacological blockade of HMGB1 reduced AV and was associated with reduced numbers of graft infiltrating Th1 and Th17 producing T cells [28]. Importantly, blockade of HMGB1 correlated with reduced numbers of inflammatory monocytes and macrophages within the spleen of cardiac allograft recipients [28]. Although not focused on AV, a recent study found that blockade of HMGB1 reduced vascular rejection and anti-donor B cells in a murine xenograft model (rat grafts into murine recipients) [29]. Overall, these studies indicate that HMGB1 enhances vascular disease and AV in mice.

Lung allografts are also susceptible to chronic allograft rejection. Chronic rejection after lung transplantation is termed bronchiolitis obliterans syndrome (BOS). Prior human studies have implicated TLR4 in the development of BOS [30]. A recent study has found that the level of hyaluronan (HA) a glycosaminoglycan that activates MyD88 [31], is increased after lung transplantation in humans and correlates with the development of BOS [32]. Interestingly, in the same study the investigators found that administration of HA broke lung transplant tolerance in mice via a MyD88-dependent process [30,32]. Other activators of innate immunity, specifically the S100 proteins or calgranulins, which can induce vascular inflammation [33] have also been found in the BAL of lung transplant recipients that exhibited signs of BOS [34]. A recent experimental study found that calgranulins were upregulated within cardiac transplants [35] but whether calgranulins associate with AV after cardiac transplantation is yet to be studied.

Complement activation as a link between IRI and AV

Activation of the complement cascade occurs in response to IRI or DSA binding to HLA molecules on endothelial cells (EC), the sites where AV lesions occur. Interestingly, a recent murine study demonstrated severe renal IRI enhanced humoral immunity [36]. Specifically, bilateral, but not unilateral renal IRI in mice, enhanced the production of antigen specific IgG₁ levels [36]. Furthermore, this study demonstrated that complement activation was critical for the amplified antigen specific humoral response induced by renal IRI as mice that are deficient in factor B, a critical component of the alternate complement cascade, exhibited an abrogated humoral response [36]. Although this study employed a native renal IRI model and not an organ transplant model, it implies the IRI after graft implantation enhances the generation of donor specific antibodies (DSA), which could enhance complement activation to promote AV.

DSA and terminal complement induce inflammatory signaling in the graft vasculature, a potential connection to AV

DSA binding to HLA I [37] and II [38] molecules on the graft vasculature potentiates the proliferative and migratory capacities of EC and promotes AV in a manner that involves

activation of Akt, a serine/threonine kinase regulating protein translation and metabolism [39]. DSA binding to HLA class I molecules via integrin $\beta 4$ activates Akt [40], which potentiates EC proliferation and migration. Underscoring its clinical significance, activated Akt phospho-targets, including S6 kinase and S6 ribosomal protein, are significantly upregulated in patients with antibody-mediated rejection [41], a clinical risk factor for development of AV. However, future studies will be required to determine if Akt activation is a target for preventing or retarding the development of AV after organ transplantation.

Complement activation occurs as a result of DSA binding to surface HLA molecules on EC and is a risk factor for cardiac AV [42]. Complement activation induces generation of anaphylatoxins C3a and C5a and assembly of membrane attack complexes (MACs) that intercalate into the surface membranes of target cells within graft tissue. Locally-produced anaphylatoxins as a result of *de novo* synthesis of alternative complement components by ECs [43] signal via G protein-coupled receptors and promote transplant rejection [44] by affecting T cell activation [45,46] and suppression [47]. Such activation could promote the development of AV.

MAC, previously believed to promote inflammation by inducing necrolysis via osmotic diffusion, similarly transmits pro-inflammatory and pro-thrombotic signals in target cells. ECs express high levels of complement regulatory proteins, in particular CD59, which, via CREB-mediated enhancement of Sp1 transcription factor activity [48], upregulates CD59 surface expression and restricts autologous MAC-induced cell death. In this context, MAC deposition on nucleated cells like EC induces cellular phenotypes that could promote AV including ERK- [49] and JNK- [50] mediated stress kinase activation, NLRP3 inflammasome activation [51, 52], and platelet activation [53,54]. Overall, these studies support the notion that MAC drives pathologic signaling processes in vascular cells that may promote AV.

By modeling the complement-activating effects of human DSA with 'high' panel reactive antibody (PRA) sera from allo-sensitized transplant candidates, we identified a novel MAC effector pathway, non-canonical NF- κ B, which was required for MAC-treated ECs to upregulate inflammatory genes and potentiate EC-mediated elaboration of IFN γ , a cytokine that promotes AV, from CD4⁺ alloimmune T cells [55], (Fig. 1). Endothelial non-canonical NF- κ B activation was furthermore detected in human coronary arteries in a humanized mouse model and in patient biopsy specimens with chronic antibody mediated rejection (CAMR) [56], a condition strongly associated with AV. Subsequent studies involving genome wide siRNA screening revealed a novel endosome-based mechanism for MAC to induce non-canonical NF- κ B activation. In this mechanism, endocytosis of MAC and the formation of MAC⁺Rab5⁺Akt⁺ vesicles were required for non-canonical NF- κ B signal activation [57]. Together, our studies show that noncanonical NF- κ B is a MAC effector pathway involved in AV requiring an endosome based signaling pathway for its activation.

Conclusions and therapeutic potential

The alarmingly high prevalence and morbidity attributable to AV underscores a generalized inefficacy of current medical therapies. As discussed above, blockade of HMGB1 in mice

reduces AV [25]. MyD88 inhibition via gene silencing enhanced cardiac allograft survival along with rapamycin treatment [34] but whether this reduced AV is unknown. Besides the MyD88 signaling pathway, complement inhibition has also emerged as a putative strategy for blocking AV lesion formation. Two recent studies targeted endogenous complement regulators CD59 [58] and Crry [59], a murine complement regulatory ortholog inhibiting C3, using a CR2 fusion protein and single chain antibody, respectively, to relevant sites *in vivo* in murine hosts to block complement activation in response to IRI. Another recent study used mAb to functionally block proximal activation with anti-C1s [60]. In addition to these antibody based therapies, pharmacologic agents blocking complement effector pathways like non-canonical NF- κ B could be explored as putative strategies for inhibiting AV. Given the paucity of new therapies to reduce the development of AV [3], clinical studies to examine if either inhibition of activators of the MyD88 pathway or the complement activation are safe and effective in reducing AV should be considered.

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Abbreviations

CAD	coronary artery disease
IRI	ischemia reperfusion injury
HMGB1	High mobility group box 1
DSA	donor-specific antibody
CAMR	Chronic Antibody-Mediated Rejection
TLR	toll-like receptor
MAC	membrane attack complex

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** Outstanding interest

* Special Interest

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Key Points

- Innate immunity and complement activation enhance AV
- MyD88 promotes AV in part via activation of HMGB1 released after graft IRI
- Non-canonical NF- κ B is a novel complement effector pathway that requires an endosome-based signaling mechanism to elicit EC activation to promote AV

Figure 1. Mechanisms of MAC Induction of ECs to enhance AV

PRA sera from allo-sensitized transplant candidates bound DSA on MHC expressed on EC, causing assembly of MAC on EC surfaces. MAC potentiated EC-mediated activation of CD4⁺IFN-γ⁺ T cells in a non-canonical NF-κB inducing kinase (NIK) dependent manner. Inflammatory cytokines produced by activated T cells (e.g., IFN-γ) enhance the development of AV.