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Recent Advances in Allograft Vasculopathy

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Abstract

Purpose of review—Despite considerable advances in controlling acute rejection, the longevity of cardiac and renal allografts remains significantly limited by chronic rejection in the form of allograft vasculopathy (AV). This review discusses recently reported mechanistic insights of AV pathogenesis as well recent clinical evaluations of new therapeutic approaches.

Recent findings—Although adaptive immunity is the major driver of AV, natural killer cells mediate vasculopathic changes in a transplanted mouse heart following treatment with donor-specific antibody (DSA). However, NK cells may also dampen chronic inflammatory responses by killing donor-derived tissue-resident CD4 T cells that provide help to host B cells, the source of DSA. DSA may directly contribute to vascular inflammation by inducing intracellular signaling cascades that upregulate leukocyte adhesion molecules, facilitating recruitment of neutrophils and monocytes. DSA-mediated complement activation additionally enhances endothelial alloimmunogenicity through activation of non-canonical NF- κ B signaling. New clinical studies evaluating mTOR and proteasome inhibitors to target these pathways have been reported.

Summary—AV is a pathology resultant from several innate and adaptive alloimmune responses. Mechanistic insights from preclinical studies have identified agents that are currently being investigated in clinical trials.

Keywords

Allograft vasculopathy; Chronic rejection; Alloimmunity; Transplantation

INTRODUCTION

Allograft vasculopathy (AV) is a major cause of late graft dysfunction following cardiac and possibly pulmonary and renal transplantation (1–3). Despite advances made in controlling acute rejection, little progress has been made in treatment or prevention of AV. Development of effective therapies for AV requires a mechanistic understanding of its underlying pathogenesis, which remains incompletely defined. The origin of graft ischemia resultant

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

from AV is distinct from other atherosclerotic conditions (such as coronary artery disease) and as such, pharmacotherapies with demonstrable efficacy in atherosclerosis have little effect in prevention of AV (4).

An understanding of AV pathogenesis has been limited in part due to a lack of relevant model systems. Data from rodent models may not accurately recapitulate human AV because AV lesions in many rodent models develop in response to acute graft vessel injury and the resulting lesions are often composed of proliferating host-derived smooth muscle cells (SMCs). In contrast, human AV lesions develop with little evidence of injury and are composed of graft-derived SMCs (5–7). Furthermore, rodent endothelium is immunologically dissimilar to human endothelium, generally lacking expression of class II MHC molecules as well as key co-stimulatory (e.g. CD58) and co-inhibitory (e.g. CD 273) molecules that regulate memory CD4+ and CD8+ T cells *in vitro* and *in vivo* (8–11). Experimental models must be shown to recapitulate features of human AV in order to define clinically relevant mechanisms. We begin this review with a brief summary defining AV and the current models of its pathogenesis, which provide context for a summary of recent studies of AV published since January 2015.

ALLOGRAFT VASCULOPATHY DEFINED

In cardiac transplantation, where AV has been most extensively analyzed, clinically significant pathology most prominently affects the epicardial and intramyocardial arteries. At these sites, diffuse stenosis due to concentric intimal expansion and inadequate compensatory (outward) remodeling results in tissue malperfusion, ischemic injury, and graft loss. The expanded intima consists of proliferating graft-derived SMCs, concentrated near the intimal-medial border and a collagen-rich extracellular matrix. The lumen of the affected arteries is lined by an intact, graft-derived endothelial cell (EC) monolayer. A host-derived mononuclear cell infiltrate, consisting primarily of T cells and macrophages, localizes sub-adjacent to the luminal ECs. Innate lymphoid cells and other myeloid cell types, such as dendritic cells, may also be present within the intima, albeit at low frequency compared to T cells and macrophages. The arterial media is largely normal in histologic appearance, whereas the adventitia may contain a chronic inflammatory infiltrate, sometimes with formation of tertiary lymphoid organs with B cell follicles (12).

CURRENT MODELS OF PATHOGENESIS

The presence of host T and B cells, the restriction of lesions to the graft vasculature, the lack of overt graft cell lysis, and a time course of development over months to years, collectively suggest that AV is primarily a consequence of chronic alloimmunity (4). Data from animal models and human tissues suggest that AV is a form of T_H1-mediated delayed-type hypersensitivity (DTH) in which interferon-gamma (IFN- γ) production by alloreactive host T cells is driven by recognition of alloantigens, especially non-self polymorphic forms of class I and II major histocompatibility complex (MHC) molecules, expressed on the surface of the graft-derived luminal ECs (4). Invasion of SMCs from the media or adventitia and local SMC proliferation likely underlie the intimal SMC accumulation. Importantly, human IFN- γ is directly mitogenic for SMCs in the intima and media of isolated human coronary

artery segments implanted as aortic interposition grafts in immunodeficient mouse hosts (13, 14). Similar results may be observed using mouse IFN- γ and wild type mouse aortic grafts implanted into IFN- γ receptor-deficient mouse hosts (15). Furthermore, many T cells identified in clinical AV lesions in the heart express IFN- γ (16, 17). IFN- γ released by intimal infiltrating T cells also induces production of chemokines that attract additional T_H1 cells and monocytes as well as SMCs (18). Additional mitogens may be produced by the infiltrates of host leukocytes or by graft cells, including ECs.

This T_H1 chronic DTH model of AV does not explain the adventitial inflammation that is linked to inadequate compensatory vascular remodeling. It also fails to explain why the majority of patients with AV will have formed donor-specific antibodies (DSA), most often complement-fixing and directed towards a non-self allele of HLA-DQ (19, 20). The link to DSA, although not detected in all patients with AV, has led some investigators to focus on the direct effects of DSA on the vessel wall as the primary driver of AV (21, 22). In support of this idea, mouse monoclonal antibody reactive with human class I MHC antigens, like the effects of IFN- γ , can produce AV-like changes in human arteries transplanted into immunodeficient mouse hosts depleted of complement (23). It is unclear in this model if the antibody causes vascular cell injury through complement activation and/or antibody-dependent cell-mediated cytotoxicity or if the intimal expansion involves recruitment of host SMCs, as occurs following allotransplantation of arterial grafts into immunocompetent mouse hosts (7). In support of a non-lytic DSA model, cultured ECs respond to these same antibodies by activating signaling pathways that induce production and/or release of SMC mitogens (24, 25). However, an alternate possibility is that DSA and sub-lytic complement contribute to a T_H1 chronic DTH-like mechanism through alterations in graft ECs that augment host T cell release of IFN- γ (4, 26).

THE ROLES OF INNATE IMMUNE CELLS IN AV

Innate immune cells, including NK cells and macrophages may serve as mediators of AV. NK cells are a subset of type 1 innate lymphoid cells, a population that produces T_H1-like cytokines, especially IFN- γ , and in the case of NK cells, may also mediate cytotoxicity. NK cells may be activated by cytokines or by engagement of germline-encoded activating receptors. Conversely, NK cells may be kept quiescent by engagement of inhibitory receptors or by regulatory T cells (T_{reg}) (27). SCID mice, which lack T and B cells, can mount a strong NK cell response against infection but do not produce AV lesions in cardiac allografts, suggesting that the effect of NK cells is dependent upon adaptive immunity (13). The role of T cells in activating NK cells can be demonstrated in a mouse heterotopic heart transplant model (28). Engagement of a DSA bound to graft ECs by NK cells expressing activating low affinity F_c receptors (CD16) has also been proposed as a mechanism of AV pathogenesis in these mouse models (29, 30).

Following F_cR engagement, macrophages may also play an important role in development of AV through release of SMC mitogens (29) and depletion of macrophages reduces AV-like lesions in mouse heterotopic heart transplants (31). Macrophages, like dendritic cells, may also release pro-inflammatory cytokines such as IL-12, which could induce type 1 innate lymphoid cells, including NK cells, as well as Th1 cells to produce IFN- γ . The presence of

macrophages within the graft is closely associated with worsened long-term outcomes in humans (32–34). Macrophages may undergo classical activation in response to T_H1 cytokines (becoming “M1” macrophages) or in response to other signals, undergoing alternative activation. Some alternatively activated states resemble macrophages found in T_H2 -type inflammatory responses (“M2” macrophages) but many other patterns of alternative activation have also been described (and often mislabeled as M2). Significantly, the presence of alternatively activated macrophages within vascular inflammatory lesions is closely correlated with progressive AV (35).

New Insights into the Roles of Innate Immune Cells in AV from Animal Models

As noted above, NK cells in Rag gene knockout animals (which, like SCID mice, lack T and B cells) can induce chronic vasculopathy in a transplanted C3H/HeJ mouse heart following treatment with anti-MHC class I antibody. A recent study has shown that NK-mediated AV in this model can be ameliorated with IFN- γ neutralization, or simultaneous disruption of perforin and Fas expression (36•). The latter finding suggests that this model may involve graft cell destruction followed by recruitment of host SMCs, which needs to be ruled out if the findings are to apply to human AV. NK cells may also play a role in damping chronic inflammatory responses by killing donor-derived tissue-resident CD4 T cells that provide help to host B cells that produce DSAs (37••). Graft-derived molecules including vanin (38) and N-octanoyl dopamine (39) may promote AV by enhancing macrophage vessel infiltration and apoptosis of SMCs, respectively.

New Insights into the Roles of Innate Immune Cells in AV from Human Tissues

Studies in lung transplant patients have demonstrated that donor-derived tissue-resident macrophages persistent within the allograft, years following transplantation and play a key role in eliciting pro-inflammatory responses to donor-specific antibody (40). NK cell-mediated chronic inflammation has further been corroborated in human tissue samples that have closely correlated inflammatory cytokines (IL-6) and markers of EC activation (VCAM-1 and ICAM-1) with AV lesions (41). Additionally, association of intragraft NK cell infiltrates with capillary inflammation and tubulitis scores assessed on renal biopsy more than one year following transplantation have been noted (42). The relationship of these microvascular changes to arterial AV is not clearly understood. It is possible that microvascular changes in lung allografts, rather than arterial changes, are the driver of late graft loss for this organ.

THE ROLES OF ADAPTIVE IMMUNE CELLS IN AV

Adaptive immunity may be defined as responses to antigens mediated by T and B cells that express somatically generated antigen receptors. Host T cell receptors for antigen may recognize non-self allelic forms of MHC molecules bound to a wide variety of different peptides (direct recognition) or may respond to peptides from non-self allelic forms of various graft proteins bound to self MHC (indirect recognition) expressed on the surface of a host antigen presenting cell. Host B cells, whose antigen receptors may recognize intact non-self MHC molecules on graft cells, can also serve as antigen presenting cells to T cells as well as sources of cytokines and of DSA. The anatomical site(s) of DSA production and

presumptive affinity maturation of DSA-producing B cells, often detected in AV lesions, is unknown (12). Adventitial B cells represent one potential site.

Many investigations have focused on the role of DSA in AV. In addition to engagement of F_C receptors on innate immune cells, DSA directly triggers inflammatory signaling in both the graft endothelium and smooth muscle and also mediates classical complement activation. Crosslinking of DSA on ECs elicits signaling pathways involved in cellular proliferation, stress fiber formation, and mitogenic signaling (43). Recent studies in humanized mouse models have revealed that direct binding of anti-HLA class I antibodies to human SMCs initiate mitogenic signaling and enhanced secretion of matrix metalloproteinases, contributing to development of AV-like lesions (44).

Complement fixation initiated by engagement of anti-HLA antibodies on EC additionally enhances graft immunogenicity. Deposition of the membrane attack complex (45) triggers non-canonical NF- κ B signaling, upregulating proteins that render the endothelium more immunogenic without directly causing cell death (26). Moreover, anaphylatoxins (bioactive complement split products), potentiate T cell activation (46), T_H1 polarization (47) and EC activation (48), though the relevance of these responses in AV has not been established.

New Insights into the Roles of Adaptive Immunity in AV from Animal Models

Studies in mouse models have affirmed the critical role donor-reactive memory CD4 T cells have in enhancing production of DSA by alloreactive B cells. In a MHC-mismatched rodent model of renal transplantation, recipients containing donor-reactive memory CD4 T cells rapidly lost allograft function in a manner dependent on B cell alloantibody generation (49). Mitigating the effects of DSA production by targeting B cells have also been attempted in mouse models. While human B cell depletion can be effectively mediated by anti-CD20 monoclonal antibody, no mouse equivalent reagent has been developed. However, mice expressing human CD20 have been generated and a recent study found that despite effective depletion of B cells (and DSA) with early anti-human CD20 antibody treatment post-transplant, a rebound of high DSA titers occurred within 1 month with development of significant interstitial graft fibrosis (50•).

New Insights into the Roles of Adaptive Immunity in AV from Studies Utilizing Human Cells and Tissues

The close association of antibody-mediated rejection with AV has been closely evaluated in explanted hearts from patients undergoing re-transplantation for failing heart allografts (51). While substantial portion of failing cardiac allografts exhibited subclinical antibody-mediated rejection, the manner in which binding of DSA promotes chronic vascular inflammation has also been the subject of recent investigation.

Anti-class I MHC antibody promotes HLA association with β_4 integrins to activate intracellular signaling cascades which in turn, upregulate leukocyte adhesion molecules, facilitating recruitment of neutrophils and monocytes (52–54). Anti-HLA-DR antibodies can increase EC secretion of IL-6, thereby amplifying a pro-inflammatory response by enhancing T_H17 expansion diminishing functional regulatory T cell (T_{reg}) proliferation (55). While classically DSA targets have been anti-HLA antibodies, the presence of non-HLA

antibodies is increasingly being recognized as correlated with chronic rejection and diminished long-term graft survival (56). However, the mechanism by which such antibodies may influence AV is unknown.

DSA-mediated activation of human SMC proliferation may also occur via generation of mitogenic sphingosine-1-phosphate (S1P) and a protective effect of an anti-S1P antibody was recently demonstrated both *in vitro* using cultured human SMCs and *in vivo* using a humanized arterial graft model (57•).

Recent work using human tissues has also elucidated the mechanism by which complement-fixing alloantibodies triggers non-canonical NF- κ B signaling in internalizing the membrane attack complex (MAC) within Rab5⁺ endosomes, forming a unique intracellular signaling complex (58). Interestingly, these effects have been shown to be attenuated with use of an anti-C5a antibody thereby implicating MAC-dependent signaling as a key mediator of endothelial activation (59••). Such findings have the potential to be translated into new treatment strategies as has recently been demonstrated the anti-C1s complement inhibitor, shown to abrogate complement-mediated activation of human aortic ECs (60••). Novel agents targeting the complement pathway are currently under investigation for use in patients at high risk for antibody-mediated rejection.

OTHER CONTRIBUTORS TO THE DEVELOPMENT OF AV

Other known risk factors for AV include acute vascular rejection, perioperative ischemia-reperfusion injury (25), or infection of the graft, particularly with CMV. Recent data has demonstrated that many of these exposures trigger innate immune pathways including non-canonical NF- κ B, high-mobility group box 1 (HMGB1), and MyD88 that enhance development of AV (61). Some investigators have proposed that AV results from the sum of these insults (“burden of injury” model) (25).

Ischemia-Reperfusion Injury

Ischemia-reperfusion injury (IRI) results from cellular necrosis and sterile inflammation following surgical implantation, often manifesting in delayed graft function (25). Complement activation is closely associated with IRI, inducing MAC formation on the surface of the graft endothelium that ultimately results in enhanced endothelial immunogenicity mediated by activation of non-canonical NF- κ B signaling. Blockade of MAC deposition through the administration of anti-C5 antibody has been recently demonstrated to result in attenuated non-canonical NF- κ B signaling and diminished formation of stenotic and thrombotic AV in human coronary arteries implanted into SCID/bg mice that had previously received adoptive transfer of human T cells allogeneic to the artery (59••).

In addition to complement-mediated IRI, recent work has demonstrated the upregulation of HMGB1 protein may further enhance endothelial activation by activation of Toll-like receptor (TLR) 4 and MyD88 signaling (62). Blockade of HMGB1 and each of these downstream effectors correlated with reduced ischemic damage, implicating its role as an enhancer of ischemia-induced vascular inflammation (63).

Cytomegalovirus Infection

Infection with cytomegalovirus (CMV) has been recognized as a significant risk factor for AV. A recent large retrospective study in patients following long-term following heart transplantation demonstrated that those with either asymptomatic or symptomatic CMV infection had significantly increased AV and reduced 10-year graft survival (64). While the exact mechanism in which CMV infection leads to AV has not been elucidated, the ability of subclinical infection to directly activate graft endothelium or enhance innate immune responses are two mechanisms which have been proposed (4). CMV readily infects ECs without causing their death, and in this manner may effectively increase CD4 T cell reactivity to the endothelium, contributing to a T_H1-like chronic DTH (65).

Immunosuppressive Pharmacotherapy

Recent reports have implicated TLR pathways in vascular injury rendered by use of chronic immunosuppressants. Calcineurin inhibitors, among the most commonly used immunosuppressants for prevention of acute rejection, contribute to the development of AV, hypertension and atherosclerosis. A recent report has shown that these activation of TLR4 by these agents contributes to the enhancement NF- κ B signaling, resulting in upregulation of adhesion molecules, chemokine expression and production of reactive oxygen species in both cultured and aortic ECs as well as vascular SMCs (66•). These findings provide mechanistic insight for vascular inflammation induced with chronic calcineurin inhibitor use and support the investigation of adjuvant therapies or calcineurin inhibitor-free regimens for maintenance immunosuppression.

NEW INSIGHTS INTO CAUSES OF AV FROM CLINICAL TRIALS

The central role of DSA in enhancing AV has led to clinical trials aimed at targeting plasma cells, a potential source of DSA not affected by B cell depletion. Recent trials with bortezomib, a proteasome inhibitor that is toxic for plasma cells, in children undergoing renal transplantation with high HLA mismatch and biopsy-proven antibody-mediated rejection was well tolerated and demonstrated stabilization of renal function (67). Larger randomized trials evaluating the effectiveness of this agent in enhancing longer-term post-transplant renal function are currently in progress (68•).

Proliferation of SMCs, like that of T cells, may be inhibited by mammalian target of rapamycin (mTOR) inhibitors, and this is the basis of their use in coated stents. More recently, it has been appreciated that these agents also affect antigen-presenting cells, including dendritic cells and vascular ECs (69). mTOR inhibitor-treated ECs lose the ability to activate IFN- γ production by alloreactive CD4⁺ T_H1 cells but retain the capacity to activate T_{regs} (70). In humanized mouse models, mTOR inhibitors can increase the ability of T_{regs} to protect human artery grafts from intimal T cell infiltration and injury (71). Everolimus, a rapamycin-like mTOR inhibitor coupled with elimination of calcineurin inhibitor immunosuppression, demonstrated a reduction in progression of AV in heart transplant patients (72•). Notably however, no effect of the NF- κ B products including IL-6 and VCAM-1 was noted in graft endothelium, suggesting that either clinical observations

may be inconsistent with mechanisms noted in human cells or that the observed clinical improvement, may in part, arise from calcineurin-inhibitor withdrawal.

CONCLUSION

Allograft vasculopathy, a form of chronic rejection, is a major cause of late graft loss, limiting the longevity of heart, and likely renal and lung allografts. Recent experimental studies have continued to explore pathogenesis using animal models as well as analyses of human cells and tissues. Such investigations support the roles of both innate and adaptive immune cells in the pathogenesis of AV. Although currently there are no effective treatments, mechanistic hypotheses arising from experimental models are currently being evaluated in clinical trials.

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KEY POINTS

- Allograft vasculopathy is a major cause of late allograft loss following for which no therapies are available
- Experimental models reproducing the salient features of human AV will increase the likelihood of uncovering clinically relevant pathogenic mechanism(s)
- Innate immunity (NK cells and donor-derived macrophages) and adaptive immunity (T cells and B cells) are implicated in AV
- Ongoing clinical trials involving proteasome and mTOR inhibitors are being evaluated for treatment and prevention of AV