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Immunological Challenges of Cardiac Transplantation: The need for better animal models to answer current clinical questions

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Abstract

In the last decade, two advances have shifted attention from cellular rejection to antibody-mediated rejection (AMR) of cardiac transplants. First, more sensitive diagnostic tests for detection of AMR have been developed. Second, improvements in immunosuppression have made severe acute cellular rejection uncommon, but have had less effect on AMR. Antibodies can contribute to graft rejection by activation of complement, by activation of vascular endothelial and smooth muscle cells, and by activation of neutrophils, macrophages or natural killer cells. Because acute rejection is a risk factor for chronic rejection in all types of organ transplants, it has been proposed that AMR can cause chronic rejection. Small animal models need to be developed to gain further insights into AMR and the role of antibodies in chronic graft arteriopathy. This article reviews the current clinical data and existing mouse models for AMR.

Keywords

Cardiac transplantation; Antibody-Mediated Rejection; C4d; Arteriopathy

Introduction

Small animal models have been instrumental in immunological advances of relevance to cardiac transplantation. Two major examples are the discovery of immunological tolerance in mice by Billingham, Brent and Medawar (1) that set the ultimate goal for all organ transplants, and the demonstration of the powerful immunosuppressive effects of cyclosporine on transplants that revived cardiac transplantation as a realistic clinical treatment (2). Continued advances in immunosuppression have made severe acute cellular rejection uncommon. This has revealed another form of rejection, namely antibody-mediated rejection (AMR), that is resistant to current immunosuppressive therapy. Although there is now general agreement that AMR is real and potentially lethal, major gaps remain in our understanding of this form of rejection, including the incidence, risk factors, diagnostic criteria, contributory mediators, tissue responses and possible chronic sequelae. This last question is of greatest concern because chronic arteriopathy remains the major barrier to long-term survival of cardiac transplants. Advanced studies with intravascular ultrasound (IVUS) of coronary arteries have

demonstrated increased intimal thickness, one of the features that define chronic arteriopathy, in about half of cardiac transplants within one year after transplantation (3). Unfortunately, current small animal models have been of limited value in examining either AMR or the role of antibodies in chronic graft arteriopathy. In this article, we will review the clinical data and critically evaluate the existing mouse models.

Antibody mediated rejection (AMR)

The largest clinical experience with AMR has been in renal allografts. Criteria for acute AMR in renal transplants were published in 2003 (4). These include circulating antibodies to donor MHC antigens, diffuse deposition of the complement split product C4d in peritubular capillaries, and morphologic evidence of acute tissue injury, such as margination of macrophages in capillaries. Using these criteria, AMR was diagnosed in 3–6% of unsensitized patients (5), and most sensitized patients (6,7). The diagnostic interpretation of C4d deposits and marginated macrophages in cardiac transplants is still debated. However, several large studies indicate that C4d is associated with donor specific antibodies and an increase risk of rejection (8–11). What additional criteria are needed to improve the specificity and sensitivity of this marker is the subject of investigation in many centers.

C4d is a product of the initial steps of the classical and lectin pathways of complement activation (Fig 1). A single C1 molecule bound to a pair of antibodies can cleave many C4 molecules. C4b, the larger split product of C4, has the unusual capacity of forming a covalent bond with nearby proteins or carbohydrates. When C4b binds to endothelial cells, it is quickly cleaved to the smaller biologically inactive C4d. This end product of C4 activation is easy to detect because it is deposited in larger quantities than antibody and it has a longer half-life (12). However, C1 and the C4 split products have limited proinflammatory effects compared to the subsequent complement components, most importantly the split products of C3 and C5. Activation of C3 and C5 produces the soluble chemotactic split products C3a and C5a in addition to the larger C3b and C5b. C3b, like C4b, can bind covalently to tissue where, in the process of regulation, it is cleaved first to iC3b and then to C3d. C5b is the first component of the membrane attack complex (MAC) that is formed by the terminal complement components. Of relevance to AMR, neutrophils and macrophages have complement receptors for C3b (CR1; CD35) and iC3b (CR3; CD11b/CD18). B cells have receptors for C3d (CR2 or CD21).

Several regulators of complement activation can terminate the complement cascade before C3 is cleaved. These include membrane bound Decay Accelerating Factor (DAF; CD 55) and membrane cofactor protein (MCP; CD46), a cofactor for factor I. DAF dissociates the C3 convertase formed by C4b and C2a as well as the subsequent C5 convertase, and MCP catalyzes cleavage of C4b and C3b to C4d and C3d, respectively. Therefore, it has been proposed that effective complement regulation of C4 may result in C4d deposits with little inflammatory response and no impairment of graft function (13,14). A disconnection of C4d from graft injury is found in some patients, who receive major blood group incompatible renal transplants under treatment with plasmapheresis and intravenous immunoglobulin (7). The term “accommodation” has been coined for this phenomenon (13). Whether accommodation occurs in cardiac transplants remains to be determined, but data have been presented to support the use of C3d in addition to C4d as a pathological marker of AMR (9,11). This raises the further possibility that upregulation of DAF, MCP or CD59 (a regulator of MAC) may cause certain cardiac transplants to be more resistant to AMR (11). Although it is not known what mechanisms control the expression of complement regulators in clinical cardiac transplants, many mediators that have been implicated in acute and chronic responses to transplants regulate DAF expression by endothelial cells in vitro. Mediators of acute inflammation, such as thrombin, TNF α and IFN γ , increase DAF expression but not CD59 or MCP expression (15, 16). The effects of cytokines on DAF expression are augmented in endothelial cells with MAC

deposited on their surface. MAC upregulation of DAF expression may represent a feedback mechanism to protect vascular integrity in sites of complement activation. Mediators associated with chronic inflammation, such as basic FGF and VEGF also upregulate the expression of DAF. The finding that cyclosporine A inhibits the upregulation of DAF expression suggests that some of the mechanisms that protect tissues from complement activation may be inhibited in transplant patients (17).

In addition to activation of complement, antibodies can modulate inflammatory responses in transplants by direct activation of vascular endothelial and smooth muscle cells as a consequence of cross-linking MHC antigens (18–20) and by activation of neutrophils, macrophages or natural killer cells through Fc receptors (21). Extensive studies by Reed and co-workers have demonstrated that even in the absence of leukocytes and complement, binding of antibodies to MHC class I antigens on endothelial cells or smooth muscle cells causes release of growth factors, upregulation of receptors and cell proliferation (18). Importantly, these proliferative responses were blocked by the mTOR inhibitor rapamycin (20). Lowenstein and his colleagues have demonstrated that antibody-mediated cross-linking of MHC class I antigens on endothelial cells can also induce exocytosis of von Willebrand factor (vWf) and P-selectin from Weibel-Palade storage granules (19). Although most of these experiments were performed on isolated endothelial cells in vitro without complement, purified components of MAC can induce endothelial cell proliferation (22) and exocytosis of vWf and P-selectin (23). Undoubtedly, these mechanisms do not occur independently in vivo, but rather interact to modulate inflammation. In vivo, non-complement activating antibodies induce the exocytosis of vWf and P-selectin followed by adhesion of platelets and neutrophils to capillaries in skin grafts, but complement activating antibodies have a more prolonged effect (24). Similarly, antibodies alone can cause endothelial cells to produce cytokines such as IL-6 and MCP-1, but increased production of these cytokines occurs when antibodies also activate macrophages through Fc receptors (21). These cytokine responses are likely enhanced further in transplanted organs by complement because macrophages express an array of receptors for complement split products.

During immune responses, complement and antibody participate in feedback loops to regulate antibody production by B cells. C3d binds to CD21, which acts as a co-receptor for the membrane Ig (mIg) antigen receptor on B cells. C3d bound to antigen has been found to increase the antibody response of B cells by lowering the threshold of concentration or affinity for an antigen to cross-link mIg (25). Antibodies bound to antigens can deliver a counterbalancing signal to B cells through the FcγRIIB for IgG. This receptor delivers an inhibitory signal to B cells and functions as a negative feedback for continued antibody production (26).

Knowledge of the mechanisms of antibody-mediated immune responses has provided insights for potential therapeutic interventions. For example, the finding that MHC cross-linking by antibody involves the mTOR pathway has obvious implications for the use of rapamycin for immunosuppression (20). Understanding feedback regulation through FcγRIIB on B cells may lead to improvements in formulations of intravenous immunoglobulin (IVIg) to inhibit alloantibody production. Ravetch and colleagues have demonstrated that the affinity of Ig binding to FcγRIIB is dependent on the carbohydrate side chain on the IgG (26). Therefore, calculated modifications of the carbohydrate side chain may increase the inhibitory effect of IVIg on B cells. The complement cascade is also a therapeutic target. Monoclonal antibodies to C5 have been used to inhibit antibody-mediated rejection (27,28). Preventing the cleavage of C5 into C5a and C5b blocks two proinflammatory links. C5a chemoattracts neutrophils and macrophages. It also activates endothelial cells as well as neutrophils and macrophages. C5b initiates the formation of MAC. Therefore, monoclonal antibodies to C5 can inhibit leukocyte and endothelial cell activation.

Chronic Graft Arteriopathy

Chronic rejection is the tissue response to the cumulative injury to a transplant over time. This includes the innate and adaptive immune responses initiated before transplantation by brain death to the donor, at the time of transplantation by ischemia-reperfusion, and after transplantation by subclinical as well as clinically apparent rejection episodes. Arteries are the most universally affected structure in all types of organ transplants. In cardiac transplants, arteriopathy of the conductance arteries is the major finding. Careful studies at the level of capillaries demonstrate a decrease in numbers ("capillary dropout"). This suggests that the capacitance vessels are also a target of the rejection process. These changes are eventually accompanied by parenchymal fibrosis.

Chronic graft arteriopathy, also known as graft vasculopathy, is characterized in cardiac transplants by a diffuse and concentric narrowing of the coronary arteries as a result of neointimal expansion that is often accompanied by adventitial fibrosis. The intimal expansion initially includes an inflammatory cell component of macrophages and T cells that then is replaced by mesenchymal cells many of which develop characteristics of smooth muscle cells. Initially, all of the smooth muscle cells were thought to originate from the media, but more recently two additional sources of these cells have been identified: circulating recipient stem cells and donor or recipient endothelial cells. In small animal models that use suboptimal levels of immunosuppression, vascular injury is extensive and recipient cells may be the major source of both endothelial and smooth muscle cells in the arterial lesions (29). However, careful studies in human cardiac transplants indicate that only about 5–10% of the endothelial cells and 2–4% of the smooth muscle cells in larger arteries are of recipient origin (30). Endothelial cell to mesenchymal cell transition also may occur under the influence of TGF β (31). Outward expansion of the artery to compensate for the thickened intima is prevented by inflammation leading to fibrosis of the adventitia. This results in decreased blood flow and ischemic changes.

Graft arteriopathy is detected clinically by radiological techniques. The International Society for Heart and Lung Transplantation (ISHLT) Registry reported that the incidence of graft arteriopathy is 7% at one year, 32% at five years, and 53% at ten years after cardiac transplantation for patients followed between 2002–2006 (32). These data are based on standard coronary angiography. Studies using more sensitive intravascular ultrasound (IVUS) detect new arteriopathy in about half of cardiac transplants within one year after transplantation (3).

Because acute rejection is a risk factor for chronic rejection in all types of organ transplants, it has been proposed that AMR can cause chronic rejection of renal transplants (33). The infrequent demonstration of C4d in renal transplant biopsies with evidence of chronic rejection has been ascribed to low levels of antibody or repeated cycles of antibody production. This is an attractive concept for cardiac transplants because antibodies and complement cause vasculocentric pathology. Moreover, many of the cells (macrophages, platelets, endothelial cells, smooth muscle cells, fibroblasts) and growth factors (basic fibroblast growth factor, platelet derived growth factor) associated with vasculopathy in transplants are stimulated by antibodies and complement (reviewed in (34)). However, direct clinical evidence for C4d in arteries of cardiac transplants is limited because large arteries are not contained in diagnostic endomyocardial biopsies. In addition, few patients have circulating antibodies to donor MHC antigens or C4d deposition on the graft at the time of explantation or death.

DNA microarray analysis provided unexpected evidence for local production of antibodies in graft arteriopathy. Microarray studies comparing the gene expression of coronaries with dilated cardiomyopathy, atherosclerosis and chronic graft arteriopathy revealed that coronaries with chronic arteriopathy had an upregulation of immunoglobulin genes including immunoglobulin

heavy chain as well as kappa and lambda light chains (34). These findings correlated with histological analyses of cardiac allografts explanted as a result of chronic arteriopathy. B cells and plasma cells were frequently found in clusters within and surrounding coronary arteries with chronic arteriopathy (Fig 2). Infiltrating plasma cells contained kappa and lambda light chains as well as all IgG subclasses.

In addition, nodules of mononuclear cells with compartmentalized T and B cell regions consistent with tertiary lymphoid neogenesis were present in approximately half of the transplants studied. Tertiary lymphoid nodules are recognized components of chronic inflammatory diseases where they are a source of local and long-lived responses to autoantigens in chronic thyroiditis and rheumatoid arthritis (35,36). B cell nodules have been frequently described in renal transplants, but their contribution to acute and chronic rejection is controversial (37).

In addition to nodules, diffuse infiltrates of B cells and plasma cells were found in the adventitia of coronaries with graft arteriosclerosis. These adventitial infiltrates were often embedded in fibrosis. Mengel and colleagues (38) have reported that transcripts associated with B cells and plasma cells are a signature of scarring in renal transplants. Recent data from experimental models indicate that B cells can promote fibrosis. For example, Sato and Tedder and co-workers (39,40) have demonstrated that bleomycin induces less fibrosis in skin and lung of CD19 deficient mice. In these experiments, CD19 deficiency also resulted in recruitment of fewer T cells, macrophages and mast cells to sites of bleomycin administration. This is of relevance because Mengel and colleagues reported that transcripts associated with mast cells were typical of fibrotic areas in renal biopsies (38). These observations suggest a potential new role for B cells in fibrogenesis that would be fruitful to explore in animal models.

Mouse Models of AMR and Chronic Arteriopathy

Experimental models that were often too reductionist to be clinically relevant convinced many basic immunologists and clinicians that mice and rats were not appropriate animals for studying AMR. Most influential were early experimental models, in which either serum or leukocytes were transferred from sensitized mice to naïve transplant recipients. These experiments demonstrated that lymphocytes could cause rejection in the absence of antibodies. Appreciation of the potential proinflammatory effects of antibodies in transplants was eroded further by experiments demonstrating that passive transfer of antibodies often prolonged graft survival in rats and mice. The opinion evolved that rats and mice had “weak” complement systems. Although some strains of mice have complement deficiencies (eg, B10.D2 old, DBA/2, AKR and A/J are C5 deficient), most mouse strains are not complement deficient. Reagents have now been developed to detect C4d and C3d in rats and mice (Fig 3). These reagents have demonstrated that rejection of cardiac transplants is frequently associated with alloantibody production and C4d and C3d deposition (12,41–44). C4d and C3d deposition is absent in cardiac allografts in immunoglobulin knock out mice and early injury to vascular endothelial cells is minimized. In some strain combinations acute rejection of MHC incompatible is also delayed in immunoglobulin knock out recipients. In these models, passive transfer of complement activating monoclonal antibodies to the donor MHC antigens restores complement deposition and accelerates graft rejection (45,46).

Induction of AMR in T and B cell immunodeficient mice (RAG^{-/-}) by passive transfer of antibodies to donor MHC antigens has been difficult to achieve. Nozaki et al (44) concluded that titer of antibody is critical to successful passive transfer of AMR to RAG^{-/-} recipients. These investigators found that wild type recipients of MHC incompatible cardiac transplants do not produce high enough titers of alloantibodies to cause AMR in passive transfer studies with RAG^{-/-} recipients. In contrast, CCR5^{-/-} mice produce titers of alloantibodies to MHC

mismatched cardiac allografts that are 15- to 25- fold higher than their wild type counterparts. Undiluted sera from CCR5^{-/-} mice containing these higher titers of alloantibodies do cause AMR in RAG^{-/-} recipients, but AMR does not result when the sera are diluted to wild type levels.

Attempts to cause AMR in RAG^{-/-} recipients with monoclonal antibodies to MHC antigens on the transplanted heart have generally produced little pathology even though they cause C4d deposition on capillaries (47,48). The use of monoclonal antibodies limits the number of epitopes recognized by the antibodies and results in lower densities of antibodies arrayed on the vessels. Whether passively transferred antibodies that cause C4d deposition in the absence of graft rejection induce graft accommodation in mice has not been explored.

The complete absence of T cells in RAG^{-/-} recipients may curtail the effects of antibodies because it is likely that antibodies and T cells have synergistic interactions. Platelets are one potential link between antibodies and T cells. Morrell et al (24) have demonstrated that antibodies to MHC antigens on endothelial cells of skin grafts cause the release of vWf and adhesion of platelets to blood vessels. Activated platelets express and secrete many factors that can localize T cells to an allograft (49,50). Release of small vasoactive molecules like adenine nucleotides (ADP and ATP), ionized calcium, histamine, serotonin and epinephrine from dense granules of activated platelets can promote leukocyte infiltration and stimulate vasoconstriction and leukocyte diapedesis. The alpha granules of platelets contain several chemokines that attract and activate macrophages and T cells, such as MIP-1 α (CCL3), RANTES (CCL5), MCP-3 (CCL7) and PF4 (CXCL4). After contact, macrophages and T cells interact with platelets through receptor/ligand pairs, including P-selectin/PSGL-1 and CD40/CD154. In the converse direction, upregulation of MHC antigens on endothelial cells by IFN γ and other cytokines secreted by T cells likely makes antibodies more effective proinflammatory mediators. These mechanisms may account for the observation by Sis et al (51) that some biopsies with genomic profiles characteristic of activated endothelial cells associated with AMR also have upregulated genes for various T cell profiles.

The use of mice to determine whether alloantibodies can contribute to chronic arteriopathy has also had limited success. Again, the use of polyclonal antibodies apparently is more effective than monoclonal antibodies. Russell and colleagues reported that passive transfer of polyclonal antibodies to SCID mice resulted in extensive arteriopathy (52). However, the same group reported that passive transfer of monoclonal antibodies to donor MHC antigens only causes small lesions at the root of the coronary arteries of cardiac transplants in RAG^{-/-} recipients (47). More widespread neointimal formation with a prominent smooth muscle cell component can be produced in cardiac allografts to rats. Both acute and chronic rejection of cardiac allografts is delayed in rats by a deficiency in C6, one of the terminal complement components that forms MAC (41,42,53).

Significant anatomical differences exist between rodent and human coronary arteries that may contribute to the apparent resistance to arteriopathy in cardiac transplants in rodents. First, the large coronary arteries in humans are located on the surface of the heart surrounded by epicardial fat. In contrast only the first few millimeters of the coronary arteries adjacent to the aortic root are on the surface of the heart in rodents. At that point the coronary arteries penetrate the myocardium. This is relevant to the development of arteriopathy because epicardial fat produces proinflammatory cytokines including IL-6, IL-8 and MCP-1 (54). The second difference is that the adventitia of the larger human coronary arteries contains vasa vasorum (55). These vessels have been implicated in the development of atherosclerosis and it is possible that they provide an "outside-in" route for the migration of inflammatory cells and cytokines into coronaries of cardiac transplants in humans but not in rodents. In addition, adult human hearts often have superimposed abnormalities before transplantation. This is most evident by

the incidence of subclinical amounts of atherosclerosis found in IVUS studies (3). Pre-existing atheroma is an indicator of more global underlying aging alterations. Moreover, these organs are often transplanted into patients who have had previous surgery (e.g., repairs of congenital abnormalities, replacement of valves, application of coronary bypass grafts, and insertion of ventricular assist devices) with ensuing inflammation, angiogenesis and scarring. In many patients, this local inflammatory environment is compounded by systemic sensitization as the result of transfusions received for the surgical procedure. In contrast to humans, the animals used as donors and recipients are usually young and healthy.

In summary, cell-mediated rejection of cardiac transplants has decreased as immunosuppressive treatment has improved. AMR and chronic arteriopathy are important causes of mortality in cardiac transplant recipients. Although diagnostic criteria have been established for acute AMR in renal transplants, much remains to be learned about AMR in cardiac transplants. Likewise, the pathogenesis of chronic arteriopathy in cardiac allografts is largely unknown. Some small animal models for acute AMR and chronic arteriopathy have been developed, but many models present perplexing idiosyncrasies.

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References

1. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature* 1953;172(4379):603–606. [PubMed: 13099277]
2. Borel JF, Meszaros J. Skin transplantation in mice and dogs. Effect of cyclosporin A and dihydrocyclosporin C. *Transplantation* 1980;29(2):161–162. [PubMed: 6986689]
3. Tuzcu EM, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J, et al. Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. *Journal of the American College of Cardiology* 2005;45(9):1538–1542. [PubMed: 15862431]
4. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, et al. Antibody-mediated rejection criteria - an addition to the banff 97 classification of renal allograft rejection. *Am J Transplant* 2003;3(6):708–714. [PubMed: 12780562]
5. Mengel M, Bogers J, Bosmans JL, Seron D, Moreso F, Carrera M, et al. Incidence of C4d stain in protocol biopsies from renal allografts: results from a multicenter trial. *Am J Transplant* 2005;5(5):1050–1056. [PubMed: 15816885]
6. Mauiyyedi S, Colvin RB. Humoral rejection in kidney transplantation: new concepts in diagnosis and treatment. *Curr Opin Nephrol Hypertens* 2002;11(6):609–618. [PubMed: 12394606]
7. Haas M, Rahman MH, Racusen LC, Kraus ES, Bagnasco SM, Segev DL, et al. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am J Transplant* 2006;6(8):1829–1840. [PubMed: 16889542]
8. Crespo-Leiro MG, Veiga-Barreiro A, Domenech N, Paniagua MJ, Pinon P, Gonzalez-Cuesta M, et al. Humoral heart rejection (severe allograft dysfunction with no signs of cellular rejection or ischemia): incidence, management, and the value of C4d for diagnosis. *Am J Transplant* 2005;5(10):2560–2564. [PubMed: 16162208]
9. Rodriguez ER, Skojec DV, Tan CD, Zachary AA, Kasper EK, Conte JV, et al. Antibody-mediated rejection in human cardiac allografts: evaluation of immunoglobulins and complement activation products C4d and C3d as markers. *Am J Transplant* 2005;5(11):2778–2785. [PubMed: 16212640]
10. Smith RN, Brousaides N, Grazette L, Saidman S, Semigran M, Disalvo T, et al. C4d deposition in cardiac allografts correlates with alloantibody. *J Heart Lung Transplant* 2005;24:1202–1210. [PubMed: 16143234]

11. Tan CD, Sokos GG, Pidwell DJ, Smedira NG, Gonzalez-Stawinski GV, Taylor DO, et al. Correlation of donor-specific antibodies, complement and its regulators with graft dysfunction in cardiac antibody-mediated rejection. *Am J Transplant* 2009;9(9):2075–2084. [PubMed: 19624562]
12. Minami K, Murata K, Lee C-Y, Fox-Talbot K, Wasowska B, Pescovitz M, et al. C4d deposition and clearance in cardiac transplants correlates with alloantibody levels and rejection in rats. *Am J Transplant* 2006;6(5):923–932. [PubMed: 16611328]
13. Platt JL. C4d and the Fate of Organ Allografts. *J Am Soc Nephrol* 2002;13:2417–2419. [PubMed: 12191987]
14. Baldwin WM III, Kasper EK, Zachary AA, Wasowska BA, Rodriguez ER. Beyond C4d: other complement related diagnostic approaches to antibody-mediated rejection. *Am J Transplant* 2004;4:311–318. [PubMed: 14961982]
15. Lidington EA, Haskard DO, Mason JC. Induction of decay-accelerating factor by thrombin through a protease-activated receptor 1 and protein kinase C-dependent pathway protects vascular endothelial cells from complement-mediated injury. *Blood* 2000;96(8):2784–2792. [PubMed: 11023512]
16. Mason JC, Yarwood H, Sugars K, Morgan BP, Davies KA, Haskard DO. Induction of decay-accelerating factor by cytokines or the membrane-attack complex protects vascular endothelial cells against complement deposition. *Blood* 1999;94(5):1673–1682. [PubMed: 10477692]
17. Mason JC, Steinberg R, Lidington EA, Kinderlerer AR, Ohba M, Haskard DO. Decay-accelerating factor induction on vascular endothelium by vascular endothelial growth factor (VEGF) is mediated via a VEGF receptor-2 (VEGF-R2)- and protein kinase C-alpha/epsilon (PKCalpha/epsilon)-dependent cytoprotective signaling pathway and is inhibited by cyclosporin A. *J Biol Chem* 2004;279(40):41611–41618. [PubMed: 15284224]
18. Bian H, Reed EF. Alloantibody-mediated class I signal transduction in endothelial cells and smooth muscle cells: enhancement by IFN-gamma and TNF-alpha. *J Immunol* 1999;163(2):1010–1018. [PubMed: 10395699]
19. Yamakuchi M, Kirkiles-Smith NC, Ferlito M, Cameron SJ, Bao C, Fox-Talbot K, et al. Antibody to human leukocyte antigen triggers endothelial exocytosis. *Proc Natl Acad Sci U S A* 2007;104(4):1301–1306. [PubMed: 17229850]
20. Jindra PT, Jin YP, Rozengurt E, Reed EF. HLA class I antibody-mediated endothelial cell proliferation via the mTOR pathway. *J Immunol* 2008;180(4):2357–2366. [PubMed: 18250445]
21. Lee C-Y, Reynolds M, Garyu J, Baldwin WM III, Wasowska BA. The involvement of FcR mechanisms in antibody-mediated rejection. *Transplantation* 2007;84:1324–1334. [PubMed: 18049118]
22. Benzaquen LR, Nicholson-Weller A, Halperin JA. Terminal complement proteins C5b-9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. *J Exp Med* 1994;179:985–992. [PubMed: 8113689]
23. Hattori R, Hamilton KK, McEver RP, Sims PJ. Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. *J Biol Chem* 1989;264(15):9053–9060. [PubMed: 2470750]
24. Morrell CN, Murata K, Swaim AM, Mason E, Martin TV, Thompson LE, et al. In vivo platelet-endothelial cell interactions in response to major histocompatibility complex alloantibody. *Circ Res* 2008;102(7):777–785. [PubMed: 18296616]
25. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science (New York, NY)* 1996;271(5247):348–350.
26. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science (New York, NY)* 2006;313(5787):670–673.
27. Wang H, Jiang J, Liu W, Kubelik D, Chen G, Gies D, et al. Prevention of acute vascular rejection by a functionally blocking anti-C5 monoclonal antibody combined with cyclosporine. *Transplantation* 2005;79(9):1121–1127. [PubMed: 15880054]
28. Wang H, Arp J, Liu W, Faas SJ, Jiang J, Gies DR, et al. Inhibition of terminal complement components in presensitized transplant recipients prevents antibody-mediated rejection leading to long-term graft survival and accommodation. *J Immunol* 2007;179(7):4451–4463. [PubMed: 17878341]

29. Xu Q. Stem cells and transplant arteriosclerosis. *Circ Res* 2008;102(9):1011–1024. [PubMed: 18467640]
30. Minami E, Laflamme MA, Saffitz JE, Murry CE. Extracardiac progenitor cells repopulate most major cell types in the transplanted human heart. *Circulation* 2005;112(19):2951–2958. [PubMed: 16275883]
31. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 2007;13(8):952–961. [PubMed: 17660828]
32. Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, Christie J, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult heart transplant report--2007. *J Heart Lung Transplant* 2007;26(8):769–781. [PubMed: 17692781]
33. Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol* 2007;18(4):1046–1056. [PubMed: 17360947]
34. Wehner J, Morrell CN, Reynolds T, Rodriguez ER, Baldwin WM 3rd. Antibody and complement in transplant vasculopathy. *Circ Res* 2007;100(2):191–203. [PubMed: 17272820]
35. Armengol MP, Juan M, Lucas-Martin A, Fernandez-Figueras MT, Jaraquemada D, Gallart T, et al. Thyroid autoimmune disease: demonstration of thyroid antigen-specific B cells and recombination-activating gene expression in chemokine-containing active intrathyroidal germinal centers. *Am J Pathol* 2001;159(3):861–873. [PubMed: 11549579]
36. Schroder AE, Greiner A, Seyfert C, Berek C. Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. *Proc Natl Acad Sci U S A* 1996;93(1):221–225. [PubMed: 8552609]
37. Zarkhin V, Li L, Sarwal M. “To B or not to B?”. B-cells and graft rejection. *Transplantation* 2008;85(12):1705–1714. [PubMed: 18580460]
38. Mengel M, Reeve J, Bunnag S, Einecke G, Sis B, Mueller T, et al. Molecular correlates of scarring in kidney transplants: the emergence of mast cell transcripts. *Am J Transplant* 2009;9(1):169–178. [PubMed: 18976290]
39. Komura K, Yanaba K, Horikawa M, Ogawa F, Fujimoto M, Tedder TF, et al. CD19 regulates the development of bleomycin-induced pulmonary fibrosis in a mouse model. *Arthritis and rheumatism* 2008;58(11):3574–3584. [PubMed: 18975313]
40. Yoshizaki A, Iwata Y, Komura K, Ogawa F, Hara T, Muroi E, et al. CD19 regulates skin and lung fibrosis via Toll-like receptor signaling in a model of bleomycin-induced scleroderma. *Am J Pathol* 2008;172(6):1650–1663. [PubMed: 18467694]
41. Qian Z, Hu W, Liu J, Sanfilippo F, Hruban RH, Baldwin WM III. Accelerated graft arteriosclerosis in cardiac transplants: complement activation promotes progression of lesions from medium to large arteries. *Transplantation* 2001;72(5):900–906. [PubMed: 11571457]
42. Qian Z, Lee CY, Murata K, Liu J, Fox-Talbot K, Wasowska BA, et al. Antibody and complement mediated injury in transplants following sensitization by allogeneic blood transfusion. *Transplantation* 2006;82(7):857–864. [PubMed: 17038898]
43. Murata K, Fox-Talbot K, Qian Z, Takahashi K, Stahl GL, Baldwin WM III, et al. Synergistic deposition of C4d by complement and non-complement activating antibodies in cardiac transplants. *Am J Transpl* 2007;7:2605–2614.
44. Nozaki T, Amano H, Bickerstaff A, Orosz CG, Novick AC, Tanabe K, et al. Antibody-mediated rejection of cardiac allografts in CCR5-deficient recipients. *J Immunol* 2007;179(8):5238–5245. [PubMed: 17911609]
45. Wasowska BA, Qian Z, Cangello DL, Behrens E, Van Tran K, Layton JL, et al. Passive transfer of alloantibodies restores acute cardiac rejection in IgKO mice. *Transplantation* 2001;71:727–736. [PubMed: 11330533]
46. Rahimi S, Qian Z, Layton J, Fox-Talbot K, Baldwin WM, Wasowska BA. Non-complement- and complement-activating antibodies synergize to cause rejection of cardiac allografts. *Am J Transplant* 2004;4(3):326–334. [PubMed: 14961984]
47. Uehara S, Chase C, Cornell L, Madsen J, Russell P, Colvin R. Chronic cardiac transplant arteriopathy in mice: Relationship of alloantibody, C4d deposition and neointimal fibrosis. *Am J Transpl* 2007;7:57–65.

48. Jindra PT, Hsueh A, Hong L, Gjertson D, Shen XD, Gao F, et al. Anti-MHC class I antibody activation of proliferation and survival signaling in murine cardiac allografts. *J Immunol* 2008;180(4):2214–2224. [PubMed: 18250428]
49. Morrell CN, Sun H, Swaim AM, Baldwin WM III. Platelets, an Inflammatory Force in Transplantation. *Am J Transplant* 2007;7:2447–2454. [PubMed: 17927608]
50. Kirk AD, Morrell CN, Baldwin WM 3rd. Platelets influence vascularized organ transplants from start to finish. *Am J Transplant* 2009;9(1):14–22. [PubMed: 19067663]
51. Sis B, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial Gene Expression in Kidney Transplants with Alloantibody Indicates Antibody-Mediated Damage Despite Lack of C4d Staining. *Am J Transplant*. 2009
52. Russell PS, Chase CM, Winn HJ, Colvin RB. Coronary atherosclerosis in transplanted mouse hearts II. Importance of humoral immunity. *J Immunol* 1994;152:5135–5141. [PubMed: 8176230]
53. Ota H, Fox-Talbot K, Hu W, Qian Z, Sanfilippo F, Hruban RH, et al. Terminal complement components mediate release of von Willebrand factor and adhesion of platelets in arteries of allografts. *Transplantation* 2005;79(3):276–281. [PubMed: 15699756]
54. Chatterjee TK, Stoll LL, Denning GM, Harrelson A, Blomkalns AL, Idelman G, et al. Proinflammatory phenotype of perivascular adipocytes: influence of high-fat feeding. *Circ Res* 2009;104(4):541–549. [PubMed: 19122178]
55. Siow RC, Churchman AT. Adventitial growth factor signalling and vascular remodelling: potential of perivascular gene transfer from the outside-in. *Cardiovascular research* 2007;75(4):659–668. [PubMed: 17678883]

Figure 1.

Classical pathway of complement activation by antibody. After the first component of complement (C1) binds to Fc portions of closely spaced antibodies, it splits C4 into C4a and C4b. C4b can bind covalently to proteins or carbohydrates on cell membranes. C2 associates with C4b and is split by C1. The complex of C2a and C3b is called the C3 convertase because it is capable of cleaving C3 into C3a and C3b. C3b, like C4b, can bind covalently to cell membranes. C3b associated with C4bC2a forms a C5 convertase that splits C5 into C5a and C5b. Neutrophils and macrophages have receptors for most of these split products (C4b, C3b, iC3b, C3a, C5a as well as the Fc segment of antibodies). C5b initiates the assembly of the terminal complement components (C6, C7, C8 and C9) into a pore-forming structure, the membrane attack complex (MAC), that can insert into a cell membrane. This cascade is regulated at several steps, especially at the C3 and C5 convertases, by membrane bound and soluble regulatory molecules. For example, decay accelerating factor (DAF; CD55) is a membrane protein that accelerates the dissociation of the convertases. This limits the amount of C3b bound to the surface of cells. Membrane co-factor protein (MCP; CD46) is a transmembrane glycoprotein that acts as a cofactor for soluble factor I to cleave C4b and C3b into enzymatically inactive iC4b and iC3b and then C4d and C3d, respectively.

Figure 2.
Coronary artery with severe graft arteriopathy contains B cells (stained brown with anti-CD20). Low power (A) shows coronary surrounded by fibrosis and epicardial fat. Medium (B) and high (C) power demonstrates B cells associated with bands of fibrosis.

Figure 3.

Immunoperoxidase stains for C4d in a cardiac allograft to a mouse (A) and rat (B). Diffuse linear deposits of C4d are present on arteries, capillaries and veins (brown). Marginated leukocytes are adherent to vascular endothelial cells (arrows).