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Mechanisms of complement activation, C4d deposition, and their contribution to the pathogenesis of antibody mediated rejection

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Abstract

Complement split products have emerged as useful markers of antibody mediated rejection in solid organ transplants. One split product, C4d, is now widely accepted as a marker for antibody mediated rejection in renal and cardiac allografts. This review summarizes the rationale for the use of C4d as a marker of antibody mediated rejection, along with the clinical evidence supporting its use in the clinical diagnosis of antibody mediated rejection. Antibody-independent mechanisms by which C4d can be activated by the classical and lectin pathways of complement activation are also identified. Finally, mechanisms by which complement activation stimulates effector cells (neutrophils, monocytes, macrophages, platelets, and B and T lymphocytes) as well as target cells (endothelial cells) are discussed in relation to antibody mediated allograft rejection.

Introduction

Transplantation is now the preferred form of treatment for end-stage cardiac and renal failure, with more than 28,000 patients receiving an organ transplant in 2006 (UNOS statistics). Numerous advances in immunosuppressive regimens have resulted in more successful stable functioning allografts. However, while organ loss due to cellular rejection is less frequent with current immunosuppressive regimens, humoral rejection, also known as antibody-mediated rejection (AMR), remains a problem in clinical transplantation, particularly in renal and cardiac transplants.

The presence of antibodies to donor HLA was initially recognized as a cause of immediate graft rejection at time of vascular reperfusion, also known as hyperacute rejection. Hyperacute rejection occurs in patients who are presensitized to donor HLA antigens through previous transplants, pregnancy, or blood transfusions. Patel and Terasaki (1) introduced the first method of cross-matching recipient sera for preformed antibodies, which virtually eliminated the risk of hyperacute rejection. However, the threat of a memory alloantibody response in presensitized patients, as well as a primary alloantibody response in unsensitized patients still remain for a subset of transplant recipients. The incidence of AMR in unsensitized patients is less than 5% (2,3), but it can reach 40 to 90% in sensitized patients (4–6). When treated with conventional therapeutic modalities, such as steroids, AMR can cause significant graft dysfunction, which may progress to graft failure. Furthermore, episodes of acute humoral

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rejection have been demonstrated to be a significant risk factor for chronic rejection in both heart (7) and kidney (8,9) transplants. While more challenging to treat relative to cell-mediated rejection, therapies such as intravenous immunoglobulin (IVIg), plasmapheresis, and anti-CD20 (rituximab) can reverse AMR. This, combined with the fact that episodes of humoral rejection places transplants at higher risk for chronic rejection, makes the early detection of AMR particularly important. Currently, immunopathologic staining of biopsy tissue for C4d, a split product of complement component 4 (C4), is a key diagnostic marker of AMR. This section will introduce the basic science behind the use of C4d as a marker for antibody-mediated rejection. Then, the correlation of C4d staining with antibody-mediated rejection in various organs, as well as the functional significance of complement deposits on allografts will be discussed.

The Biological basis for the use of C4d as a marker for AMR

C4d was initially proposed as a marker for humoral rejection by Feucht et al (10,11), who demonstrated the correlation between C4d deposition on peritubular capillaries (PTC) in renal allograft biopsies and poor clinical outcome, and later suggested that alloantibodies may be responsible for the pathology (12). The rationale for the selection of C4d as a marker for antibody mediated rejection comes from its position in the complement cascade. There are three main pathways for complement activation, the classical, lectin, and alternative pathways. Of these, only the classical and lectin pathways are known to involve C4.

C4 is a glycoprotein with an approximate molecular weight of 210 kDa, and is present in the serum in quantities of approximately 600 µg/ml. It is comprised of three subunits (α , β , and γ) with molecular weights of 90 kDa, 80 kDa, and 30 kDa respectively, linked together by disulfide bonds. C4d is a split product of C4 that is generated in the process of complement activation and subsequent regulation of the biologically active C4 split products.

Activation of C4 by the classical pathway

Antibodies of the IgM or IgG class are the most efficient means of activating the classical pathway of complement. The complement component C1q binds to structures in two or more Fc domains of IgM or IgG. This causes C1q to undergo a conformational change, which allows the enzymatic components C1r and C1s in the collagenous portion of the antibody-bound C1q to cleave C4 molecules. The cleavage of C4 by the C1r/C1s complex results in the formation of two split fragments: fluid phase C4a, a small fragment, which diffuses away, and a large fragment, C4b. The cleavage of C4 results in the exposure of an internal thiolester group in the α -chain portion of C4b, which remains highly reactive for approximately a few microseconds, during which time it is able to form a covalent bond with a nearby protein or carbohydrate through formation of either an ester or amide linkage. As a result of this mechanism, C4b can bind covalently to the antibody or the surface of a cell such as a vascular endothelial cell. Alternatively, in the absence of encountering a suitable substrate within a brief duration of time, the binding site is lost through the spontaneous hydrolysis by surrounding water. This results in the formation of soluble C4b, which, like C4a, diffuses away in blood or interstitial fluids. It is estimated that approximately 95% of activated C4 ends up as soluble C4b depending upon the conditions under which C4b is generated (13). C4b serves as a ligand for C2 that then is in position to be cleaved by C1s into a soluble split fragment called C2b and C2a that remains complexed with C4b to form a C3 convertase (C4bC2a). This C3 convertase cleaves C3 into fluid phase C3a and C3b, which can form covalent bonds in a homologous manner to C4b. With the addition of C3b to the C4bC2a complex, a C5 convertase is formed that can then cleave C5 into C5a and C5b. C5b is the first component of the membrane attack complex (MAC) that is composed of the terminal components of the complement cascade (Figure 1).

Several regulatory mechanisms have evolved to protect autologous tissues against complement mediated lysis. Regulatory mechanisms can be grouped into two categories: disassociation and cleavage. Most of the regulatory molecules accelerate the normal disassociation of non-covalently complexed complement components. For example, Decay Accelerating Factor (DAF/CD55) is a membrane bound protein that accelerates the disassociation of C4b and C2a. In contrast, factor I is the only regulator of complement that functions by cleaving the active split products of C4 and C3 into inactive fragments. C4b bound to cells may be further cleaved by factor I into an enzymatically inactive form of C4b, called inactive C4b, or iC4b. This fragment is referred to as “inactive” due to its inability to function as part of the C3 convertase. Further cleavage of iC4b by factor I results in two fragments, soluble C4c, and surface-bound C4d (Figure 2). It is this surface bound molecule, which currently serves as a marker for AMR. To date, no biological function has been attributed to C4d, and no receptors have been identified. C4d is an attractive marker for complement activation because it binds tissue close to the site of activation and the covalent bond does not break spontaneously. It should also be noted that one C1 molecule is able to cleave enough C4 to cause deposition of approximately 25 molecules of C4b (14) before finally being inactivated by C1 inhibitor (C1 INH), which dissociates the C1r and C1s from C1q. This amplification is responsible for the increased sensitivity of C4d in the detection of AMR.

Other mechanisms of C4 activation

The mannose binding lectin (MBL) pathway is another pathway by which C4, can be activated. MBL is a serum protein highly analogous to C1q, composed of multiple copies of a 32kDa chain. Structurally, MBL resembles C1q, with 4 to 6 copies of the 32kDa chain forming a multimeric molecule with globular binding regions and a collagenous stalk. The globular heads are able to bind to terminal mannose as well as N-acetyl glucosamine residues that are present on bacterial and yeast cell walls. After binding of the globular heads to multiple sugar residues, MBL associates with serine proteases homologous to C1r and C1s, referred to as MBL Associated Serine Proteases (MASPs), specifically MASPs-1, 2, and 3, to activate complement in a manner similar to the C1 complex. This results in the cleavage and binding of C4 in a process identical to that described previously. Alternatively, MBL has also been shown to activate C3 directly when bound to certain bacteria, bypassing the cleavage of C4 (15,16). Although the lectin pathway of complement activation was not conventionally considered to contribute to C4d deposition in AMR, MBL recently has been demonstrated to contribute significantly to complement activation in a murine model of AMR (17).

IgM is known to activate complement efficiently through the classical pathway. Due to its pentameric structure, single IgM molecules are able to activate complement, unlike IgG, which requires at least two molecules. However, activation of complement first requires IgM to bind antigen and undergo conformational changes that expose the C1q binding sites (18). IgM has recently been reported to be able to activate complement through the MBL pathway (19). One study reported that MBL preferentially bound the hexameric form rather than the pentameric form of IgM (20).

IgA does not activate complement by the classical pathway due to its inability to bind C1q. However, when complexed with an antigen, polymeric IgA has been shown to activate complement through the alternative pathway (21–23), and degalactosylated IgA can activate complement by the lectin pathway (24).

Glycosylation variants, known as glycoforms, of IgG have also been shown to activate complement, not only through the classical pathway, but also through the MBL pathway. Glycoforms of IgG lacking a terminal galactose residue in the Fc region of the IgG molecule, known as G0 glycoforms are elevated in the serum of patients with rheumatoid arthritis. MBL

can bind to the exposed N-acetyl glucosamine (GlcNAc) on the G0 isoforms of IgG and activate complement (25).

Other pattern recognition proteins besides MBL have been shown to activate complement. C-reactive protein (CRP) is an acute phase protein belonging to the pentraxin family. CRP has been reported to facilitate clearance of apoptotic cells by binding to phosphatidylserine molecules on the inner leaflet of the “flipped” membrane and activate the classical pathway of complement through C1q binding (26). On the other hand, CRP has also been reported to induce apoptosis of endothelial cells (27).

C1q can also cause C4d deposition on apoptotic cells. It was initially shown that C1q could bind to apoptotic blebs on keratinocytes (28), and later on blebs from apoptotic vascular endothelial cells (29). This binding of C1q to apoptotic blebs results in complement activation (30) and clearance of apoptotic cells without inducing inflammation.

Correlation of C4d deposition with circulating alloantibodies

The diagnosis of antibody mediated rejection in renal transplants is currently based on criteria established during the Sixth Banff Conference on Allograft Pathology in 2001 (31). These criteria include the three following cardinal features:

1. Morphologic evidence of acute tissue injury
2. Immunopathologic staining for C4d in peritubular capillaries
3. Presence of circulating antibodies to donor HLA or other antigens expressed on donor endothelial cells.

Numerous studies support the use of these criteria, and have documented correlations between C4d staining and the presence of circulating donor-specific antibodies (DSA) and allograft rejection. Mauiyyedi et al have concluded from a review of 232 kidney transplants that C4d deposition was associated with neutrophils in the peritubular capillaries, glomeruli, and tubules, as well as fibrinoid necrosis and acute tubular injury (32). Moreover, serum from 90% of patients with C4d in their grafts tested positive for DSA as measured by cell cytotoxicity assays or flow cytometry. Those transplants diagnosed with AMR based on the pathologic criteria mentioned above had a graft loss rate of 30% at 1 year versus 4% for acute cellular rejection. Koo et al have reported similar findings in a study of 48 patients (33). C4d deposition was present in 13% of all biopsies in the study, and DSA were found in all recipients that were positive for C4d. In addition, C4d deposition was found in 33% of biopsies with acute rejection compared to only 3% in biopsies with no rejection.

For cardiac transplants, the diagnostic criteria for AMR have been established by the International Society for Heart and Lung Transplantation (ISHLT) in 2004 (34). These include the following:

1. Swelling or denudation of capillaries
2. Presence of macrophages and/or neutrophils in capillaries, interstitial edema and/or hemorrhage and fibrin in vessels.

The presence of any of these pathological changes in endomyocardial biopsy justifies further confirmation through immunohistological staining of tissue for macrophages in capillaries as well as C4d.

The correlation between C4d staining in endomyocardial biopsies and the presence of DSA has been demonstrated in a study by Smith et al (35). They found that 21 of 25 (84%) biopsy samples from patients whose sera tested positive for DSA stained positive for C4d, while 53

of 60 (88%) samples without antibody did not have demonstrable C4d, indicating that C4d staining is both a sensitive and a specific test for the presence of alloantibodies.

In contrast to renal and cardiac transplants, antibody mediated rejection of lung transplants has only recently been recognized, and is still somewhat controversial. Studies to date examining the use of C4d as a marker for lung allograft rejection have yielded conflicting results. The first clinical studies of C4d in lung transplants reported widespread staining on structures beyond the vasculature (36,37). In addition, the pattern was reported as granular and sometimes nuclear. This pattern is different than the diffuse, linear pattern of C4d deposition localized to vascular endothelial cells that is usually described in renal or cardiac transplants (31,³⁸,39). The C4d deposits in lung transplants were associated with septal capillary necrosis and deposition of IgG, C1q, C3, and or C5b-9. These lung transplant recipients were distinguished from renal or cardiac transplant recipients with AMR by the fact that none of them had detectable antibodies to HLA in their circulation by ELISA and bead-based flow cytometry. Instead, it was suggested by the authors that non-HLA antigens on endothelial cells were the antigenic target of the antibodies, based on positive granular nuclear staining of fixed human umbilical vein cells by serum obtained from a patient during a rejection episode. A subsequent study by Ionescu et al (40) described subendothelial deposition of C4d that correlated with the presence of donor-specific HLA antibodies in the serum of the recipient. All patients with biopsies that were positive for C4d had detectable circulating donor-specific HLA antibodies, but only 31% of patients testing positive for donor-specific alloantibodies had subendothelial deposition of C4d. Thus, the investigators suggested that C4d is a specific, but not very sensitive marker for antibody-mediated rejection. Miller and colleagues (41) measured C4d levels in serial bronchoalveolar lavage (BAL) samples from a small group of patients and found that elevated C4d levels were correlated with antibodies to HLA. In contrast, Wallace et al (42) studied 68 lung transplant biopsies and concluded that C4d staining of paraffin-embedded allograft biopsies does not identify acute, chronic, or humoral rejection in lung allograft tissue. Although the authors reported that sera from many of these patients were screened for the presence of antibodies to HLA, no data was presented on whether they correlated with C4d staining.

Recently, the ISHLT issued a consensus statement which included a discussion of acute antibody-mediated rejection of the lung (43). Based on the available literature, including the studies discussed above, as well as extrapolation from data available from kidney and heart transplantation, the group suggested that immunologic staining for C4d may be of limited clinical use in protocol biopsies due to the patchy nature and low sensitivity of C4d staining in the lung. Although the possibility of its use as a marker of co-existent AMR in patients with refractory acute cellular rejection was discussed, no definitive recommendations were made by the group regarding the diagnosis or treatment of antibody mediated rejection in the lung.

In more controlled experimental models of orthotopic lung transplants in inbred rats, the correlation between alloantibody responses and C4d deposition is more easily appreciated. In these models, C4d deposition in the transplanted lungs was coincident with the presence of circulating donor specific IgM and IgG alloantibodies in the recipients. C4d deposition was initially detected as an intense, diffuse, continuous linear staining pattern on the vascular endothelium in rejecting lung allografts, while no staining was detectable in control isografts or native lungs (Murata et al, in press). Perivascular accumulation of macrophages and T cells, as well as neutrophils in the intravascular and alveolar capillary compartments were also noted. The diverse findings of these studies clearly support the need for larger studies on C4d as a potential marker for AMR in the lung including biopsy specimens from multiple transplant centers, as well as further investigations in animal models of lung transplantation.

Correlation between C4d deposition and rejection: The need for co-markers

While there is good correlation between the presence of C4d staining and circulating alloantibodies, the presence of C4d alone or alloantibodies alone are insufficient to make a diagnosis of AMR. The Banff criteria for the diagnosis of AMR in renal transplants require both the presence of circulating antibodies in addition to morphologic evidence of tissue injury along with C4d deposition, while ISHLT guidelines for cardiac transplants also require changes in the capillary endothelium as well as the presence of macrophages, neutrophils, edema, or hemorrhage.

The original complement dependent cytotoxic assays on leukocytes for alloantibodies have been supplanted by enzyme linked or flow cytometry based assays. These newer assays have increased sensitivity, and, when isolated antigens are used as targets instead of whole cells, specificity. In addition, the newer assays can be used to identify IgM and IgA as well as IgG subclasses of alloantibodies. However, unless subclasses of IgG are identified, these tests do not differentiate between complement fixing and non-complement fixing alloantibodies. This is a crucial distinction, as complement activation has been shown to significantly accelerate rejection of cardiac (44) and lung (45) transplants. In humans, 4 different IgG subclasses, IgG1, IgG2, IgG3, and IgG4 have been identified, which differ greatly in their ability to activate complement. IgG3 is the most efficient activator of complement, followed by IgG1 and IgG2. IgG4 lacks the ability to activate complement. Differences in complement activation are partly attributable to differences in affinity of C1q for these antibodies (46). It has been shown in kidney transplants that the detection of anti-HLA antibodies by Flow Panel Reactive Antibodies (FlowPRA) do not necessarily correlate with positive C4d staining in the biopsied tissue (47). Furthermore, superior clinical outcomes were reported for patients that were C4d negative, yet positive for antibodies, whereas patients that were positive for both C4d and antibodies had significantly worse outcomes. This illustrates the impact of complement activation by alloantibodies on graft survival as well as the need to discriminate between the presence of complement-fixing vs. non-complement fixing antibodies. To address this problem, Wahrmann et al have recently developed a technique that allows detection of antibody-mediated activation of complement by measuring deposition of C4d on the surface of microparticles coated with HLA antigens (48).

However, while *in vitro* methods may detect the presence of alloantibodies with varying sensitivity and specificity, all of these approaches fail to assess specifically the damaging effect of antibodies on local tissue, which, currently can only be confirmed through tissue biopsies. The need to correlate *in vitro* findings with tissue biopsies is highlighted particularly in ABO incompatible renal transplants, where the presence of alloantibodies have not always been correlated with pathological signs of rejection in tissue specimens, in a phenomenon referred to as transplant accommodation. Transplant accommodation was initially identified in ABO blood group incompatible renal transplants (49,50), and refers to a condition in which an organ transplant functions normally despite the presence of donor-specific antibodies in the recipient, and C4d deposition in the graft. These grafts lack other histological features of AMR such as cellular infiltration or tissue damage. Although accommodation is relatively frequent in blood group incompatible transplants, it is less common when donor specific antibodies to HLA are detected (51).

Chemoattraction and stimulation of effector cells by anaphylatoxins

Although C4d is functionally inactive and only serves as a useful marker for AMR, complement activation can account for the accumulation of neutrophils, monocytes and macrophages that are characteristic of AMR. C5a is known to be the most potent chemotactic split product of complement activation (52). The effects of C5a on neutrophils and other cells are mediated

through the C5a receptor (53,54). A second receptor, C5L2, has been identified and shown to bind C5a with high affinity. Originally thought to have no biological function due to its lack of coupling to any known G-proteins (55), C5L2 has now been shown to be crucial for optimal C5a and C3a signaling (56). Cells known to be chemoattracted by C5a include neutrophils (57,58), monocytes, and macrophages (59). These cell types are seen frequently in biopsies of organs undergoing AMR. The influx of neutrophils into lung allograft tissue is associated with the development of acute (60) as well as chronic (61,62) rejection, and the presence of macrophages in biopsies is now included as one of the diagnostic criteria for humoral rejection in cardiac (34) and kidney (63) transplants.

In addition to its chemoattractive properties, C5a is known to directly induce proinflammatory responses in neutrophils, including stimulation of an oxidative burst and enhancement of phagocytosis (64) as well as the release of granule enzymes (65,66). Moreover, C5a has been shown to modulate cytokine expression, inhibit neutrophil apoptosis, enhance the expression of neutrophil adhesion proteins, and activate the coagulation pathway (67–70). C5a also increases CD11b/CD18 expression on neutrophils, inducing increased adhesion of neutrophils to endothelial cells through iC3b and ICAM-1 (71,72).

Anaphylatoxins also have significant effects on endothelial cells. C5a has been shown to upregulate expression of adhesion molecules, including E-selectin, ICAM-1, and VCAM-1, as well as various cytokines and chemokines and their related receptors, such as VEGF, IL-6, and IL-18(73). Although not as potent as C5a, C3a can upregulate IL-8, IL-1 β , and RANTES expression in endothelial cells (74).

In recent years, humanized monoclonal antibodies to C5 with the ability to block cleavage of C5 into its constitutive C5a and C5b fragments have been developed (eculizumab and pexelizumab, Alexion Pharmaceuticals). These antibodies have been evaluated for their clinical efficacy in a variety of disorders, including paroxysmal nocturnal hemoglobinuria (75), ischemia-reperfusion injury during cardiac bypass surgery (76–78), and myocardial infarction (79–81). In murine models of cardiac and renal allografts (82,83), antibody to C5 has been found to be effective in preventing acute rejection when combined with low doses of cyclosporine. Decreased macrophage infiltrates was the primary change in pathology caused by antibody to C5. Antibodies to C5 are being introduced clinically as a potential treatment for AMR. Small molecule inhibitors for C5a and soluble C5a receptor are under development and may provide additional treatment options in the future. It is possible that these treatments may lower the future risk of developing chronic rejection by reducing the level of inflammation caused by chronic, low levels of antibody induced complement activation.

Surface bound split products of complement and their interaction with phagocytic cells

Cells that have been initially drawn to the site of complement activation undergo further signaling by cell bound complement split products, such as C4b, iC4b, C3b, iC3b, and antibodies, all of which may be found on the surface of vascular endothelium in organs undergoing AMR. Macrophages as well as neutrophils possess receptors for these complement split products, most notably complement receptors 1 and 3 (CR1 and CR3), also known as CD35 and CD11b/CD18, respectively.

Although CR1 is expressed constitutively in low levels on monocytes and macrophages, it can be upregulated tenfold by exposure to anaphylatoxins. CR1 has multiple functions, including the regulation of complement activation by serving as a cofactor for factor I, which breaks C3b down into its enzymatically inactive form, iC3b. As a receptor, CR1 binds preferentially to

C3b and C4b, and is involved in neutrophil, but not macrophage phagocytosis (84) of opsonized particles and pathogens.

CR3, a member of the integrin family, recognizes iC3b, and perform functions related to phagocytosis, leukocyte trafficking and migration, synapse formation, and costimulation. CR3 is expressed on neutrophils, monocytes, macrophages, and on subsets of lymphocytes. CR3 expression is upregulated upon stimulation by cytokines, and it initiates pro-inflammatory signaling upon binding to its ligand, iC3b (85). Although CR3 has been shown to bind particles opsonized by iC3b *in vitro* (86), *in vivo* studies have documented that CR3 contributes indirectly to clearance of iC3b coated pathogens by recruitment of neutrophils through ICAM-1 expressed on the surface of neutrophils (87–89). Engagement of CR3 is also associated with a systemic inflammatory response, which can affect donor responses to allografts.

CR1g is the most recent complement receptor to be discovered (90,91), and belongs to the immunoglobulin superfamily. CR1g expression is restricted to a subset of tissue resident macrophages including liver Kupffer cells, interstitial macrophages in heart, synovial lining macrophages in joint, and foam cells in atherosclerotic plaques (86) (92) (91). Human CR1g is also highly expressed on Hofbauer cells in placenta, adrenal gland macrophages, and alveolar macrophages (86). CR1g binds primarily to beta chain of C3b (93), and appears to contribute not only to the recognition of complement-opsonized particles, but also in the active phagocytosis of such particles (86).

Although the exact role of phagocytes in the pathogenesis of AMR is still unclear, stimulated macrophages have been shown to contribute to lung allograft rejection through the release of proinflammatory molecules (94,95). Macrophages have also been shown to generate high levels of IL-6 in combination with endothelial cells in an FcR dependent manner in a mouse model of AMR (96). Blocking the influx of neutrophils into the graft can also suppress allograft rejection in animal models of heart (97) and lung (60) transplantation. Phagocytes may also contribute to AMR by taking up opsonized cellular debris and apoptotic cells and presenting donor antigens, thereby initiating and/or perpetuating an alloimmune antibody response. Macrophages have long been known to be efficient professional antigen presenting cells (APC). Neutrophils are also capable of presenting phagocytized antigen (98).

Activation of endothelial cells by MAC

The vascular endothelium, which serves as the first interactive barrier between host elements and the graft, as well as the surface upon which complement is predominantly deposited during AMR, is profoundly affected by complement activation. The terminal components of the complement cascade in particular have been shown to have a variety of effects on endothelial cells. The formation of even sublytic quantities of MAC on cells results in an influx of calcium as well as activation of phospholipases and mitogen-activated protein kinase cascades (99–101), which can result in the activation of endothelial cells. This activation of endothelial cells through MAC insertion has various consequences, including upregulation of surface expression of P-selectin, an adhesion molecule, which aids in the adhesion of inflammatory cells to the injured endothelium (102). MAC insertion also induces the formation of intracellular gaps (103), resulting in the extravasation of cells from the vascular compartment into the graft tissue. Activation of endothelial cells by complement further promotes the attraction and stimulation of other immune effector cells to the site of inflammation through the upregulation of various immune modulators such as tissue factor (104), COX-2 (105), IL-8 and MCP-1 (106). In addition to these effects, insertion of sublytic levels of MAC can result in the release of von Willebrandt factor (vWf) and P-selectin (CD62P) from granules known as Weibel-Palade bodies in endothelial cells (107). This activation of platelets by vWf results in the aggregation of platelets in the region surrounding vWf release, and further release of

additional vWf from the α storage granules in platelets. The involvement of MAC in the intravascular release of vWf and aggregation of platelets in organ allografts has been demonstrated by experiments in C6 deficient rats (45,¹⁰⁸,109). The C6 deficiency prevents assembly of MAC and decreases vWF release, platelet aggregation and graft rejection.

The interplay between complement and platelets in graft rejection

As mentioned above, complement activation may also contribute to graft rejection through its effects on platelets, a cell whose role in graft rejection has, until recently, been long ignored. Although intravascular aggregates of platelets have been noted in clinical and experimental models of antibody-mediated rejection (45,¹⁰⁸–111), mechanistic studies on the role of platelets in inflammation have been largely confined to their function in the development of vasculitis and atherosclerosis. Known primarily for their role in the maintenance of vascular hemostasis, platelets also contain a multitude of pro-inflammatory mediators such as chemokines, adhesion molecules, vasoactive mediators, costimulatory molecules, and growth factors. These mediators, packaged in storage granules in platelets, can be released rapidly upon platelet activation, and may play critical roles in allograft rejection. Complement activation has been shown to cause both platelet aggregation and activation, and may also be partly responsible for the aggregation of platelets seen in allografts. This can occur through exposure of platelets to the anaphylatoxin C3a (112) or through insertion of MAC into the platelet membrane (113). Once activated, platelets may add to the exocytosis of P-selectin and vWf from endothelial cells (114), thereby concentrating vWf to the site of vascular inflammation and further localizing aggregates of platelets through interactions with GPIb (115). Activated platelets also release various chemokines such as RANTES and MCP-3 that arrest monocytes at sites of platelet deposition (116), as well as platelet factor 4 (PF4) and β -thromboglobulin (117), which recruit neutrophils. This, along with the chemotactic and ligand functions of complement split products, may further contribute to the presence of macrophages and neutrophils seen in allografts with AMR.

Conversely, platelet activation also influences complement activation. Activated platelets release casein kinase, which phosphorylates C3b, leading to delay in cleavage inactivation of C3b (118,119) contributing to prolonged complement activation. Furthermore, C3b binds to the P-selectin expressed on activated platelets and initiates the alternative pathway of complement activation (120). Platelets granules also contain significant quantities of complement proteins, including precursors of C3 and C4 (121). Thus, the effects of complement activation on graft rejection can be further enhanced by activated platelets, resulting in a positive feedback loop.

Direct visualization of platelet reactions in transplants has been reported by Morrell et al, who demonstrated that alloantibodies induce sustained platelet rolling on vascular endothelial cells in a murine model of skin transplantation. Platelet rolling was accompanied by vascular pathology, including vWf release, formation of platelet aggregates and C4d deposition. In addition, platelets were found to sustain leukocyte-endothelial interactions in vivo (122). Thus, complement activation and the activation of platelets appear to be mechanistically closely interrelated, and may play even greater roles in allograft rejection than previously thought.

Complement, B cells, and antibody production

In addition to modulating innate immunity, complement modulates B and T cell functions. C3d, the split product of C3, has been shown to have a significant effect on B cells that are the effector cells responsible for generating the humoral response. C3d deposits are frequently found coincident with C4d deposits in AMR, and can be associated with more severe rejection in cardiac and renal transplantation (2,5). Although C4d is inactive, its homologue, C3d, is the primary ligand for complement receptor 2 (CR2/CD21). In early studies, Melches et al showed

that cross linked but not soluble human C3d stimulates proliferation of activated murine B cells (123). These effects were later discovered to be mediated through the stimulation of B cells through CR2 (124,125). The most elegant experiments demonstrating a role for C3d in augmenting the humoral response were conducted by Dempsey et al, who found that coupling C3d to HEL significantly lowered amount of antigen required to activate B cells (126). CR2 signaling has also been found to promote the development of optimal B-cell memory (127). The activation of complement and subsequent deposition of C3d on donor antigens such as HLA molecules on the endothelial surface may serve to stimulate B cells and amplify a pre-existing antibody response that results in a positive feedback loop. This may partly account for local B cell proliferation and antibody production. Experiments by Marsh et al have shown that C3 deficient mice have an impaired IgG antibody response to allogeneic skin transplants as well as prolonged survival of their skin grafts (128). Clinical evidence supporting a role for surface bound complement in perpetuating a local humoral response in acute AMR comes from a study of biopsies of renal transplants where C4d deposition in peritubular capillaries was correlated with the presence of plasma cell-rich infiltrates and poor clinical outcome (129). Although no studies to date have directly examined the role of C3d in the pathogenesis of AMR, this is one area that deserves further scrutiny.

Complement modifies antigen presentation and T cell responses

Recently, several studies have demonstrated that C3 and C5 modify T cell responses to antigen presentation. In contrast to the effects of complement discussed in the previous sections, T cell responses appear to be largely modified by locally produced C3 and C5. A critical role for locally produced C3 in T cell responses to transplants was initially indicated by the finding that renal allografts from C3 deficient mice demonstrated prolonged graft survival (130), which was accompanied by decreased T cell function. Subsequent experiments demonstrated that dendritic cells and T cells from C3 deficient mice showed impaired immune responses in vitro (131,132). Although not all of the mechanisms involved in the effects of complement on T cell responses have not been elucidated, it appears that production of C3 and C5 by dendritic cells results in the binding of C3 split products on these cells. Experiments with T cells from DAF deficient mice indicate that the expression of DAF on T cells regulates T cell responses to alloantigens, and that these effects are mediated through the generation of C5a which binds to C5a receptors (133). These findings demonstrate another interface between innate and adaptive immunity, as well as a potential novel role for complement in the pathogenesis of AMR. Efficient antigen presentation, along with T-cell help, are crucial to the development of an effective antibody response.

Conclusion

Although the complement split product C4d is now used widely as a pathological marker of AMR, there is much to be learned about the effects of complement activation on rejection processes. While the chemotactic and stimulatory effects of complement split products on neutrophils and macrophages have been well-established, the more recent findings regarding the impact of complement on platelet interactions with endothelial cells and leukocytes, as well as the regulatory effects of complement on B and T cells, add new dimensions to the influence of innate immunity on adaptive immune responses. All of these facets of complement biology are highly relevant to the pathogenesis of acute and chronic rejection of organ allografts. Future research may reveal further insights into both the diagnostic and mechanistic implications of complement activation on organ rejection.

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References

1. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *The New England journal of medicine* 1969;280:735–739. [PubMed: 4886455]
2. Rodriguez ER, Skojec DV, Tan CD, 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;5:2778–2785. [PubMed: 16212640]
3. Mengel M, Bogers J, Bosmans JL, et al. Incidence of C4d stain in protocol biopsies from renal allografts: results from a multicenter trial. *Am J Transplant* 2005;5:1050–1056. [PubMed: 15816885]
4. Colvin RB, Smith RN. Antibody-mediated organ-allograft rejection. *Nat Rev Immunol* 2005;5:807–817. [PubMed: 16175181]
5. Haas M, Rahman MH, Racusen LC, et al. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am J Transplant* 2006;6:1829–1840. [PubMed: 16889542]
6. Casarez TW, Perens G, Williams RJ, et al. Humoral rejection in pediatric orthotopic heart transplantation. *J Heart Lung Transplant* 2007;26:114–119. [PubMed: 17258143]
7. Michaels PJ, Espejo ML, Kobashigawa J, et al. Humoral rejection in cardiac transplantation: risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. *J Heart Lung Transplant* 2003;22:58–69. [PubMed: 12531414]
8. Mauiyyedi S, Pelle PD, Saidman S, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 2001;12:574–582. [PubMed: 11181806]
9. David-Neto E, Prado E, Beutel A, et al. C4d-positive chronic rejection: a frequent entity with a poor outcome. *Transplantation* 2007;84:1391–1398. [PubMed: 18091514]
10. Feucht HE, Felber E, Gokel MJ, et al. Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. *Clinical and experimental immunology* 1991;86:464–470. [PubMed: 1747954]
11. Feucht HE, Schneeberger H, Hillebrand G, et al. Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney international* 1993;43:1333–1338. [PubMed: 8315947]
12. Feucht HE, Opelz G. The humoral immune response towards HLA class II determinants in renal transplantation. *Kidney Int* 1996;50:1464–1475. [PubMed: 8914011]
13. Morgan, BP. *Complement: Clinical Aspects and Relevance to Disease*. San Diego: Academic Press Inc.; 1990.
14. Ollert MW, Kadlec JV, David K, Petrella EC, Bredehorst R, Vogel CW. Antibody-mediated complement activation on nucleated cells. A quantitative analysis of the individual reaction steps. *J Immunol* 1994;153:2213–2221. [PubMed: 8051421]
15. Schweinle JE, Ezekowitz RA, Tenner AJ, Kuhlman M, Joiner KA. Human mannose-binding protein activates the alternative complement pathway and enhances serum bactericidal activity on a mannose-rich isolate of *Salmonella*. *The Journal of clinical investigation* 1989;84:1821–1829. [PubMed: 2592561]
16. Matsushita M, Fujita T. Cleavage of the third component of complement (C3) by mannose-binding protein-associated serine protease (MASP) with subsequent complement activation. *Immunobiology* 1995;194:443–448. [PubMed: 8749236]
17. Murata K, Fox-Talbot K, Qian Z, et al. Synergistic deposition of C4d by complement-activating and non-activating antibodies in cardiac transplants. *Am J Transplant* 2007;7:2605–2614. [PubMed: 17868071]
18. Perkins SJ, Nealis AS, Sutton BJ, Feinstein A. Solution structure of human and mouse immunoglobulin M by synchrotron X-ray scattering and molecular graphics modelling. A possible mechanism for complement activation. *Journal of molecular biology* 1991;221:1345–1366. [PubMed: 1942055]
19. McMullen ME, Hart ML, Walsh MC, Buras J, Takahashi K, Stahl GL. Mannose-binding lectin binds IgM to activate the lectin complement pathway in vitro and in vivo. *Immunobiology* 2006;211:759–766. [PubMed: 17113913]

20. Chan RK, Ibrahim SI, Takahashi K, et al. The differing roles of the classical and mannose-binding lectin complement pathways in the events following skeletal muscle ischemia-reperfusion. *J Immunol* 2006;177:8080–8085. [PubMed: 17114482]
21. Daha MR, Gorter A, Rits M, van Es LA, Hiemstra PS. Interaction of immunoglobulin A with complement and phagocytic cells. *Progress in clinical and biological research* 1989;297:247–260. discussion 260–241. [PubMed: 2587587]
22. Rits M, Hiemstra PS, Bazin H, Van Es LA, Vaerman JP, Daha MR. Activation of rat complement by soluble and insoluble rat IgA immune complexes. *European journal of immunology* 1988;18:1873–1880. [PubMed: 3220102]
23. Lucisano Valim YM, Lachmann PJ. The effect of antibody isotype and antigenic epitope density on the complement-fixing activity of immune complexes: a systematic study using chimaeric anti-NIP antibodies with human Fc regions. *Clinical and experimental immunology* 1991;84:1–8. [PubMed: 1707767]
24. Teraï I, Kobayashi K, Vaerman JP, Mafune N. Degalactosylated and/or denatured IgA, but not native IgA in any form, bind to mannose-binding lectin. *J Immunol* 2006;177:1737–1745. [PubMed: 16849483]
25. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nature medicine* 1995;1:237–243.
26. Ciurana CL, Hack CE. Competitive binding of pentraxins and IgM to newly exposed epitopes on late apoptotic cells. *Cell Immunol* 2006;239:14–21. [PubMed: 16643876]
27. Nabata A, Kuroki M, Ueba H, et al. C-reactive protein induces endothelial cell apoptosis and matrix metalloproteinase-9 production in human mononuclear cells: Implications for the destabilization of atherosclerotic plaque. *Atherosclerosis* 2008;196:129–135. [PubMed: 17531242]
28. Korb LC, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. *J Immunol* 1997;158:4525–4528. [PubMed: 9144462]
29. Navratil JS, Watkins SC, Wisniewski JJ, Ahearn JM. The globular heads of C1q specifically recognize surface blebs of apoptotic vascular endothelial cells. *J Immunol* 2001;166:3231–3239. [PubMed: 11207277]
30. Nauta AJ, Trouw LA, Daha MR, et al. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. *European journal of immunology* 2002;32:1726–1736. [PubMed: 12115656]
31. Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria - an addition to the banff 97 classification of renal allograft rejection. *Am J Transplant* 2003;3:708–714. [PubMed: 12780562]
32. Mauiyyedi S, Crespo M, Collins AB, et al. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol* 2002;13:779–787. [PubMed: 11856785]
33. Koo DD, Roberts IS, Quiroga I, et al. C4d deposition in early renal allograft protocol biopsies. *Transplantation* 2004;78:398–403. [PubMed: 15316368]
34. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005;24:1710–1720. [PubMed: 16297770]
35. Smith RN, Brousaides N, Grazette L, et al. C4d deposition in cardiac allografts correlates with alloantibody. *J Heart Lung Transplant* 2005;24:1202–1210. [PubMed: 16143234]
36. Magro CM, Deng A, Pope-Harman A, et al. Humorally mediated posttransplantation septal capillary injury syndrome as a common form of pulmonary allograft rejection: a hypothesis. *Transplantation* 2002;74:1273–1280. [PubMed: 12451265]
37. Magro CM, Klinger DM, Adams PW, et al. Evidence that humoral allograft rejection in lung transplant patients is not histocompatibility antigen-related. *Am J Transplant* 2003;3:1264–1272. [PubMed: 14510700]
38. Feucht HE. Complement C4d in graft capillaries -- the missing link in the recognition of humoral alloreactivity. *Am J Transplant* 2003;3:646–652. [PubMed: 12780555]
39. Fishbein MC, Kobashigawa J. Biopsy-negative cardiac transplant rejection: etiology, diagnosis, and therapy. *Current opinion in cardiology* 2004;19:166–169. [PubMed: 15075746]

40. Ionescu DN, Girit AL, Zeevi A, et al. C4d deposition in lung allografts is associated with circulating anti-HLA alloantibody. *Transplant immunology* 2005;15:63–68. [PubMed: 16223674]
41. Miller GG, Destarac L, Zeevi A, et al. Acute humoral rejection of human lung allografts and elevation of C4d in bronchoalveolar lavage fluid. *Am J Transplant* 2004;4:1323–1330. [PubMed: 15268735]
42. Wallace WD, Reed EF, Ross D, Lassman CR, Fishbein MC. C4d staining of pulmonary allograft biopsies: an immunoperoxidase study. *J Heart Lung Transplant* 2005;24:1565–1570. [PubMed: 16210131]
43. Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007;26:1229–1242. [PubMed: 18096473]
44. Wasowska BA, Qian Z, Cangello DL, et al. Passive transfer of alloantibodies restores acute cardiac rejection in IgKO mice. *Transplantation* 2001;71:727–736. [PubMed: 11330533]
45. Nakashima S, Qian Z, Rahimi S, Wasowska BA, Baldwin WM 3rd. Membrane attack complex contributes to destruction of vascular integrity in acute lung allograft rejection. *J Immunol* 2002;169:4620–4627. [PubMed: 12370401]
46. Morgan, BP.; Harris, CL. Complement regulatory proteins. San Diego: Academic Press; 1999.
47. Bohmig GA, Exner M, Habicht A, et al. Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol* 2002;13:1091–1099. [PubMed: 11912271]
48. Wahrman M, Exner M, Regele H, et al. Flow cytometry based detection of HLA alloantibody mediated classical complement activation. *Journal of immunological methods* 2003;275:149–160. [PubMed: 12667679]
49. Bennett AD, McAlack RF, Raja R, Baquero A, Morris M. Experiences with known ABO-mismatched renal transplants. *Transplantation proceedings* 1987;19:4543–4546. [PubMed: 3321615]
50. Bennett AD, McAlack RF, Morris M, Chopek MW, Platt JL. ABO incompatible renal transplantation: a qualitative analysis of native endothelial tissue ABO antigens after transplantation. *Transplantation proceedings* 1989;21:783–785. [PubMed: 2650269]
51. Racusen LC, Haas M. Antibody-mediated rejection in renal allografts: lessons from pathology. *Clin J Am Soc Nephrol* 2006;1:415–420. [PubMed: 17699240]
52. Snyderman R, Pike MC, McCarley D, Lang L. Quantification of mouse macrophage chemotaxis in vitro: role of C5 for the production of chemotactic activity. *Infect Immun* 1975;11:488–492. [PubMed: 46841]
53. Gerard NP, Hodges MK, Drazen JM, Weller PF, Gerard C. Characterization of a receptor for C5a anaphylatoxin on human eosinophils. *The Journal of biological chemistry* 1989;264:1760–1766. [PubMed: 2912983]
54. Gerard NP, Gerard C. The chemotactic receptor for human C5a anaphylatoxin. *Nature* 1991;349:614–617. [PubMed: 1847994]
55. Okinaga S, Slatery D, Humbles A, et al. C5L2, a nonsignaling C5A binding protein. *Biochemistry* 2003;42:9406–9415. [PubMed: 12899627]
56. Chen NJ, Mirtsos C, Suh D, et al. C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. *Nature* 2007;446:203–207. [PubMed: 17322907]
57. Becker EL. The relationship of the chemotactic behavior of the complement-derived factors, C3a, C5a, and C567, and a bacterial chemotactic factor to their ability to activate the proesterase 1 of rabbit polymorphonuclear leukocytes. *The Journal of experimental medicine* 1972;135:376–387. [PubMed: 4551218]
58. Snyderman R, Phillips J, Mergenhagen SE. Polymorphonuclear Leukocyte Chemotactic Activity in Rabbit Serum and Guinea Pig Serum Treated with Immune Complexes: Evidence for C5a as the Major Chemotactic Factor. *Infect Immun* 1970;1:521–525. [PubMed: 16557770]
59. Marder SR, Chenoweth DE, Goldstein IM, Perez HD. Chemotactic responses of human peripheral blood monocytes to the complement-derived peptides C5a and C5a des Arg. *J Immunol* 1985;134:3325–3331. [PubMed: 3884709]
60. Hirayama, S.; Shiraishi, T.; Shirakusa, T.; Higuchi, T.; Miller, EJ. *Molecular medicine*. Vol. 12. Cambridge, Mass: 2006. Prevention of neutrophil migration ameliorates rat lung allograft rejection; p. 208-213.

61. DiGiovine B, Lynch JP 3rd, Martinez FJ, et al. Bronchoalveolar lavage neutrophilia is associated with obliterative bronchiolitis after lung transplantation: role of IL-8. *J Immunol* 1996;157:4194–4202. [PubMed: 8892657]
62. Hirsch J, Elssner A, Mazur G, et al. Bronchiolitis obliterans syndrome after (heart-)lung transplantation. Impaired antiprotease defense and increased oxidant activity. *Am J Respir Crit Care Med* 1999;160:1640–1646. [PubMed: 10556134]
63. Fahim T, Bohmig GA, Exner M, et al. The cellular lesion of humoral rejection: predominant recruitment of monocytes to peritubular and glomerular capillaries. *Am J Transplant* 2007;7:385–393. [PubMed: 17283488]
64. Mollnes TE, Brekke OL, Fung M, et al. Essential role of the C5a receptor in E coli-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 2002;100:1869–1877. [PubMed: 12176911]
65. Goldstein IM, Weissmann G. Generation of C5-derived lysosomal enzyme-releasing activity (C5a) by lysates of leukocyte lysosomes. *J Immunol* 1974;113:1583–1588. [PubMed: 4473046]
66. Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. *The Journal of clinical investigation* 1978;61:1161–1167. [PubMed: 207729]
67. Riedemann NC, Guo RF, Sarma VJ, et al. Expression and function of the C5a receptor in rat alveolar epithelial cells. *J Immunol* 2002;168:1919–1925. [PubMed: 11823527]
68. Guo RF, Riedemann NC, Laudes IJ, et al. Altered neutrophil trafficking during sepsis. *J Immunol* 2002;169:307–314. [PubMed: 12077259]
69. Laudes IJ, Chu JC, Sikrath S, et al. Anti-c5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. *The American journal of pathology* 2002;160:1867–1875. [PubMed: 12000738]
70. Laudes IJ, Chu JC, Huber-Lang M, et al. Expression and function of C5a receptor in mouse microvascular endothelial cells. *J Immunol* 2002;169:5962–5970. [PubMed: 12421982]
71. Molad Y, Haines KA, Anderson DC, Buyon JP, Cronstein BN. Immunocomplexes stimulate different signalling events to chemoattractants in the neutrophil and regulate L-selectin and beta 2-integrin expression differently. *Biochem J* 1994;299 (Pt 3):881–887. [PubMed: 7514872]
72. Jagels MA, Daffern PJ, Hugli TE. C3a and C5a enhance granulocyte adhesion to endothelial and epithelial cell monolayers: epithelial and endothelial priming is required for C3a-induced eosinophil adhesion. *Immunopharmacology* 2000;46:209–222. [PubMed: 10741901]
73. Albrecht EA, Chinnaiyan AM, Varambally S, et al. C5a-induced gene expression in human umbilical vein endothelial cells. *The American journal of pathology* 2004;164:849–859. [PubMed: 14982839]
74. Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine MC. Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. *Faseb J* 2003;17:1003–1014. [PubMed: 12773483]
75. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *The New England journal of medicine* 2006;355:1233–1243. [PubMed: 16990386]
76. Fitch JC, Rollins S, Matis L, et al. Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation* 1999;100:2499–2506. [PubMed: 10604887]
77. Verrier ED, Shernan SK, Taylor KM, et al. Terminal complement blockade with pexelizumab during coronary artery bypass graft surgery requiring cardiopulmonary bypass: a randomized trial. *Jama* 2004;291:2319–2327. [PubMed: 15150203]
78. Shernan SK, Fitch JC, Nussmeier NA, et al. Impact of pexelizumab, an anti-C5 complement antibody, on total mortality and adverse cardiovascular outcomes in cardiac surgical patients undergoing cardiopulmonary bypass. *Ann Thorac Surg* 2004;77:942–949. discussion 949–950. [PubMed: 14992903]
79. Armstrong PW, Granger CB, Adams PX, et al. Pexelizumab for acute ST-elevation myocardial infarction in patients undergoing primary percutaneous coronary intervention: a randomized controlled trial. *Jama* 2007;297:43–51. [PubMed: 17200474]

80. Granger CB, Mahaffey KW, Weaver WD, et al. Pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to primary percutaneous coronary intervention in acute myocardial infarction: the COMplement inhibition in Myocardial infarction treated with Angioplasty (COMMA) trial. *Circulation* 2003;108:1184–1190. [PubMed: 12925454]
81. Mahaffey KW, Granger CB, Nicolau JC, et al. Effect of pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to fibrinolysis in acute myocardial infarction: the COMplement inhibition in myocardial infarction treated with thromboLYtics (COMPLY) trial. *Circulation* 2003;108:1176–1183. [PubMed: 12925455]
82. Wang H, Jiang J, Liu W, et al. Prevention of acute vascular rejection by a functionally blocking anti-C5 monoclonal antibody combined with cyclosporine. *Transplantation* 2005;79:1121–1127. [PubMed: 15880054]
83. Wang H, Arp J, Liu W, 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;179:4451–4463. [PubMed: 17878341]
84. Sengelov H, Kjeldsen L, Kroeze W, Berger M, Borregaard N. Secretory vesicles are the intracellular reservoir of complement receptor 1 in human neutrophils. *J Immunol* 1994;153:804–810. [PubMed: 8021514]
85. Xia Y, Vetvicka V, Yan J, Hanikyrova M, Mayadas T, Ross GD. The beta-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J Immunol* 1999;162:2281–2290. [PubMed: 9973505]
86. Helmy KY, Katschke KJ Jr, Gorgani NN, et al. CR1g: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell* 2006;124:915–927. [PubMed: 16530040]
87. Rogers HW, Unanue ER. Neutrophils are involved in acute, nonspecific resistance to *Listeria monocytogenes* in mice. *Infect Immun* 1993;61:5090–5096. [PubMed: 8225586]
88. Gregory SH, Cousens LP, van Rooijen N, Dopp EA, Carlos TM, Wing EJ. Complementary adhesion molecules promote neutrophil-Kupffer cell interaction and the elimination of bacteria taken up by the liver. *J Immunol* 2002;168:308–315. [PubMed: 11751975]
89. Conlan JW, North RJ. Neutrophils are essential for early anti-*Listeria* defense in the liver, but not in the spleen or peritoneal cavity, as revealed by a granulocyte-depleting monoclonal antibody. *The Journal of experimental medicine* 1994;179:259–268. [PubMed: 8270870]
90. Langnaese K, Colleaux L, Kloos DU, Fontes M, Wieacker P. Cloning of Z39Ig, a novel gene with immunoglobulin-like domains located on human chromosome X. *Biochim Biophys Acta* 2000;1492:522–525. [PubMed: 11004523]
91. Vogt L, Schmitz N, Kurrer MO, et al. VSIG4, a B7 family-related protein, is a negative regulator of T cell activation. *The Journal of clinical investigation* 2006;116:2817–2826. [PubMed: 17016562]
92. Lee MY, Kim WJ, Kang YJ, et al. Z39Ig is expressed on macrophages and may mediate inflammatory reactions in arthritis and atherosclerosis. *J Leukoc Biol* 2006;80:922–928. [PubMed: 16882875]
93. Wiesmann C, Katschke KJ, Yin J, et al. Structure of C3b in complex with CR1g gives insights into regulation of complement activation. *Nature* 2006;444:217–220. [PubMed: 17051150]
94. Nicod LP, Joudrier S, Isler P, Spiliopoulos A, Pache JC. Upregulation of CD40, CD80, CD83 or CD86 on alveolar macrophages after lung transplantation. *J Heart Lung Transplant* 2005;24:1067–1075. [PubMed: 16102442]
95. Frachon I, Fattal-German M, Magnan A, et al. Emergence of inflammatory alveolar macrophages during rejection or infection after lung transplantation. *Transplantation* 1994;57:1621–1628. [PubMed: 7516588]
96. Lee CY, Lotfi-Emran S, Erdinc M, et al. The involvement of FcR mechanisms in antibody-mediated rejection. *Transplantation* 2007;84:1324–1334. [PubMed: 18049118]
97. El-Sawy T, Belperio JA, Strieter RM, Remick DG, Fairchild RL. Inhibition of polymorphonuclear leukocyte-mediated graft damage synergizes with short-term costimulatory blockade to prevent cardiac allograft rejection. *Circulation* 2005;112:320–331. [PubMed: 15998678]
98. Ashtekar AR, Saha B. Poly's plea: membership to the club of APCs. *Trends Immunol* 2003;24:485–490. [PubMed: 12967672]

99. Carney DF, Hammer CH, Shin ML. Elimination of terminal complement complexes in the plasma membrane of nucleated cells: influence of extracellular Ca²⁺ and association with cellular Ca²⁺. *J Immunol* 1986;137:263–270. [PubMed: 3711667]
100. Cybulsky AV, Bonventre JV, Quigg RJ, Lieberthal W, Salant DJ. Cytosolic calcium and protein kinase C reduce complement-mediated glomerular epithelial injury. *Kidney international* 1990;38:803–811. [PubMed: 2266662]
101. Kraus S, Fishelson Z. Cell desensitization by sublytic C5b-9 complexes and calcium ionophores depends on activation of protein kinase C. *European journal of immunology* 2000;30:1272–1280. [PubMed: 10820372]
102. Foreman KE, Vaporciyan AA, Bonish BK, et al. C5a-induced expression of P-selectin in endothelial cells. *J Clin Invest* 1994;94:1147–1155. [PubMed: 7521884]
103. Saadi S, Platt JL. Transient perturbation of endothelial integrity induced by natural antibodies and complement. *The Journal of experimental medicine* 1995;181:21–31. [PubMed: 7807003]
104. Saadi S, Holzknicht RA, Patte CP, Stern DM, Platt JL. Complement-mediated regulation of tissue factor activity in endothelium. *J Exp Med* 1995;182:1807–1814. [PubMed: 7500026]
105. Bustos M, Coffman TM, Saadi S, Platt JL. Modulation of eicosanoid metabolism in endothelial cells in a xenograft model. Role of cyclooxygenase-2. *The Journal of clinical investigation* 1997;100:1150–1158. [PubMed: 9276732]
106. Kilgore KS, Flory CM, Miller BF, Evans VM, Warren JS. The membrane attack complex of complement induces interleukin-8 and monocyte chemoattractant protein-1 secretion from human umbilical vein endothelial cells. *Am J Pathol* 1996;149:953–961. [PubMed: 8780399]
107. Hattori R, Hamilton KK, McEver RP, Sims PJ. Complement proteins C5b–C9 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;264:9053–9060. [PubMed: 2470750]
108. Qian Z, Pfaff-Ameese T, Behrens E, et al. C6 produced by macrophages contributes to cardiac allograft rejection. *Transplantation* 1998;65:S77.
109. Ota H, Fox-Talbot K, Hu W, et al. Terminal complement components mediate release of von Willebrand factor and adhesion of platelets in arteries of allografts. *Transplantation* 2005;79:276–281. [PubMed: 15699756]
110. Halloran PF, Wadgymar A, Ritchie S, Falk J, Solez K, Srinivasa NS. The significance of the anti-class I antibody response: I. Clinical and pathological features of anti-class I-mediated rejection. *Transplantation* 1990;49:85–91. [PubMed: 2301035]
111. Meehan SM, Limsrichamrern S, Manaligod JR, et al. Platelets and capillary injury in acute humoral rejection of renal allografts. *Hum Pathol* 2003;34:533–540. [PubMed: 12827606]
112. Polley MJ, Nachman RL. Human platelet activation by C3a and C3a des-arg. *The Journal of experimental medicine* 1983;158:603–615. [PubMed: 6604123]
113. Sims PJ, Faioni EM, Wiedmer T, Shattil SJ. Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *The Journal of biological chemistry* 1988;263:18205–18212. [PubMed: 2848029]
114. Dole VS, Bergmeier W, Mitchell HA, Eichenberger SC, Wagner DD. Activated platelets induce Weibel-Palade-body secretion and leukocyte rolling in vivo: role of P-selectin. *Blood* 2005;106:2334–2339. [PubMed: 15956287]
115. Padilla A, Moake JL, Bernardo A, et al. P-selectin anchors newly released ultralarge von Willebrand factor multimers to the endothelial cell surface. *Blood* 2004;103:2150–2156. [PubMed: 14630802]
116. von Hundelshausen P, Weber KS, Huo Y, et al. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. *Circulation* 2001;103:1772–1777. [PubMed: 11282909]
117. Weiss HJ, Witte LD, Kaplan KL, et al. Heterogeneity in storage pool deficiency: studies on granule-bound substances in 18 patients including variants deficient in alpha-granules, platelet factor 4, beta-thromboglobulin, and platelet-derived growth factor. *Blood* 1979;54:1296–1319. [PubMed: 508939]

118. Ekdahl KN, Nilsson B. Alterations in C3 activation and binding caused by phosphorylation by a casein kinase released from activated human platelets. *J Immunol* 1999;162:7426–7433. [PubMed: 10358196]
119. Nilsson-Ekdahl K, Nilsson B. Phosphorylation of C3 by a casein kinase released from activated human platelets increases opsonization of immune complexes and binding to complement receptor type 1. *European journal of immunology* 2001;31:1047–1054. [PubMed: 11298329]
120. Del Conde I, Cruz MA, Zhang H, Lopez JA, Afshar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. *The Journal of experimental medicine* 2005;201:871–879. [PubMed: 15781579]
121. Coppinger JA, Cagney G, Toomey S, et al. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood* 2004;103:2096–2104. [PubMed: 14630798]
122. Morrell CN, Murata K, Swaim AM, et al. In Vivo Platelet-Endothelial Cell Interactions in Response to Major Histocompatibility Complex Alloantibody. *Circulation research*. 2008
123. Melchers F, Erdei A, Schultz T, Dierich MP. Growth control of activated, synchronized murine B cells by the C3d fragment of human complement. *Nature* 1985;317:264. [PubMed: 3876511]
124. Matsumoto AK, Kopicky-Burd J, Carter RH, Tuveson DA, Tedder TF, Fearon DT. Intersection of the complement and immune systems: a signal transduction complex of the B lymphocyte-containing complement receptor. *J Exp Med* 1991;173:55.
125. Bradbury LE, Kansas GS, Levy S, Evans RL, Tedder TF. The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. *J Immunol* 1992;149:2841–2850. [PubMed: 1383329]
126. Dempsey PW, Allison MED, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: Bridging innate and acquired immunity. *Science* 1996;271:348–350. [PubMed: 8553069]
127. Molina H, Holers VM, Li B, et al. Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93:3357–3361. [PubMed: 8622941]
128. Marsh JE, Farmer CK, Jurcevic S, Wang Y, Carroll MC, Sacks SH. The allogeneic T and B cell response is strongly dependent on complement components C3 and C4. *Transplantation* 2001;72:1310–1318. [PubMed: 11602861]
129. Desvaux D, Le Gouvello S, Pastural M, et al. Acute renal allograft rejections with major interstitial oedema and plasma cell-rich infiltrates: high gamma-interferon expression and poor clinical outcome. *Nephrol Dial Transplant* 2004;19:933–939. [PubMed: 15031352]
130. Pratt JR, Basheer SA, Sacks SH. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nature medicine* 2002;8:582–587.
131. Peng Q, Li K, Patel H, Sacks SH, Zhou W. Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J Immunol* 2006;176:3330–3341. [PubMed: 16517700]
132. Heeger PS, Lalli PN, Lin F, et al. Decay-accelerating factor modulates induction of T cell immunity. *The Journal of experimental medicine* 2005;201:1523–1530. [PubMed: 15883171]
133. Lalli PN, Strainic MG, Lin F, Medof ME, Heeger PS. Decay accelerating factor can control T cell differentiation into IFN-gamma-producing effector cells via regulating local C5a-induced IL-12 production. *J Immunol* 2007;179:5793–5802. [PubMed: 17947652]

Complement Cascade

Fig 1.

The complement cascade can be activated through three different initial pathways: the classical (C1, C4 and C2), alternative (factor B), and mannose binding lectin (MBL) pathways. All three pathways converge in the activation of C3. Activation of C3 and C5 occurs by enzymatic cleavage into two biologically active fragments. The small fragments (C3a and C5a) mediate chemotaxis and activation through separate receptors (C3aR and C5aR) on leukocytes. Activation of monocytes through their C5a receptor causes production of proinflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF α . The large fragment of C3 can bind covalently to proteins or carbohydrates. Deposition of C3b can be increased through an amplification loop

with factor B of the alternative pathway. The terminal components of complement (C5b-C9) form a tubular structure, the membrane attack complex (MAC).

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C4d is the end product of C4 regulation by factor I

Fig 2.

C4 is activated by enzymatic cleavage into C4a and C4b that can bind covalently to proteins or carbohydrates through a thioester bond. C4b is cleaved successively by factor I leaving first iC4b and then C4d bound to the membrane. CR1 or factor H serve as co-factors to Factor I. C4b and iC4b stimulate cells with complement receptors CR1 or CR3, respectively. C4d has no known receptor.

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