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Effects of complement activation on allograft injury

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Abstract

Purpose of review—To summarize the current knowledge regarding mechanisms linking the complement system to transplant injury, highlighting findings reported since 2013.

Recent findings—Building upon the documentation that complement activation is a pathogenic mediator of post-transplant ischemia-reperfusion (IR) injury, emerging evidence indicates blocking either the classical or lectin pathways attenuates IR injury in animal models. Immune cell-derived and locally activated complement, including intracellular C3 positively modulates allo-reactive T cell activation and expansion, while simultaneously inhibiting regulatory T cell induction and function, together promoting transplant rejection. While alloantibody-initiated complement activation directly injures target cells, complement-dependent signals activate endothelial cells to facilitate T cell dependent inflammation. Complement activation within allografts contributes to progressive chronic injury and fibrosis.

Summary—The complement cascade, traditionally considered relevant to transplantation only as an effector mechanism of antibody-initiated allograft injury, is now understood to damage the allograft through multiple mechanisms. Complement activation promotes post-transplant IR injury, formation and function of allo-antibody, differentiation and function of alloreactive T cells, and contributes to chronic progressive allograft failure. The recognition that complement impacts transplant injury at many levels provides a foundation for targeting complement as a therapy to prolong transplant survival and improve patient health.

Keywords

complement; T cells; antibody mediated rejection; ischemia reperfusion

INTRODUCTION

The complement system is traditionally considered a component of the innate immune system. In the context of transplantation, complement activation is a well-recognized effector mechanism underlying alloantibody mediated rejection (1, 2). Evidence published since the late 1990s has expanded our understanding of complement's role in allograft injury. Complement participates in the pathogenesis of ischemia-reperfusion (IR) injury,

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modulates alloreactive T cell immunity, and contributes to chronic allograft failure. Herein we will summarize the current state of knowledge regarding complement and transplant injury, highlighting new findings published since 2013.

OVERVIEW OF COMPLEMENT

The complement system is comprised of soluble and membrane-bound proteins, including zymogens, receptors and regulators that link innate and adaptive immunity. The complement cascade, outlined in Figure 1, can be activated via the lectin pathway, the classical pathway, and the alternative pathway (3). Convergence at a central amplification step forms multimeric C3 convertases (3) which cleave C3 to C3a and C3b, the latter initiating formation of the C5 convertase and, ultimately, the membrane attack complex (MAC, C5b-9). Soluble and surface bound split products, including C3a, C3b, iC3b, C3dg and C5a mediate inflammation by directly lysing target cells, serving as chemoattractants, functioning as opsonins, and activating innate immune cells, including macrophages and neutrophils (4). Regardless of the initiating pathway, cascade amplification predominantly occurs at the C3 convertase step and is driven by the alternative pathway (Figure 1). Circulating/systemic complement proteins are produced by the liver but complement proteins are also produced by tissue-resident [*e.g.* tubular cells in the kidney (5)] and migratory/immune cells, including T cells and antigen presenting cells [APCs (6)]. Under physiological conditions, complement activation is highly regulated by several membrane-bound and soluble regulatory proteins to prevent injury to self-cells (4) (see Figure 1). The protective effects of complement regulators can be overcome under pathogenic conditions although precise mechanisms remain to be elucidated.

COMPLEMENT AND ISCHEMIA-REPERFUSION INJURY

Post-transplant ischemia induces tissue hypoxia, mitochondrial damage and ATP depletion, followed by the generation of free oxygen radicals and endothelial damage upon reperfusion (7). Subsequent inflammation is partially dependent upon complement activation (8–11). Work performed in murine kidney transplant models revealed that donor kidney-derived C3, and not systemic recipient C3, is the predominant complement source driving IR injury (12). Data from animal models and humans suggest that donor brain death upregulates complement activation in the donor kidney prior to organ removal (13). The mechanisms through which complement mediates IR injury include signals transmitted via C3a/C5a interactions with their receptors, C3aR/C5aR (14), including (but not limited to) C3a/C3aR dependent production of chemokines by renal tubular epithelial cells (15). The complement-dependent inflammation associated with IR injury can amplify adaptive alloimmunity (16) and can facilitate T cell infiltration into the allograft (17, 18), together potentially resulting in negative long-term consequences to the transplanted organ (Figure 2A).

Understanding the signals that initiate complement activation following post-transplant IR injury has the potential to guide development of preventative therapies. A 2013 publication showed significantly reduced kidney injury in mannan-binding lectin serine peptidase 2 (MASP2)-deficient mice implicating the lectin pathway (19). Cardiac IR injury also results in mannose lectin pathway-dependent complement activation initiated by binding of natural

IgM reactive to tissue-expressed neo-antigens (including non-muscle myosin heavy chain II) that are upregulated/exposed by hypoxia (20, 21). Complement activation via the classical pathway also contributes to murine liver IR injury (22). Blocking complement activation with recombinant C1-INH (inhibits C1qrs, Figure 1) was effective in preventing IR injury in an animal model (23). Therapeutic use of C1-INH improved survival and oxygenation in lung transplant patients with early signs of primary graft dysfunction (24) supporting the need to more broadly test the efficacy of this agent to prevent IR injury in human transplant recipients.

Regardless of the activation pathway, amplification of the complement cascade initiated by IR is alternative pathway-dependent (Figure 1) and results in deposition of C3b on the ischemic graft cells (8–11). To target this mechanism, one research team conjugated a human complement-regulatory protein CD35 (complement receptor 1, CR1, binds to C3b/C4b and blocks complement activation at the C3 convertase step, Figure 1) to a myristoylated peptidyl tail such that when administered by intravenous perfusion of the harvested organ ex vivo it self-inserts into the lipid bilayer of the endothelial cell membranes (25). The approach inhibited local complement activation and limited post-transplant kidney IR injury in rats (26). A human version, mirococept (APT070), is being tested for its efficacy to prevent post-transplant DGF (25). Murine kidney IR injury was analogously prevented through treatment with a protein comprised of the complement regulator C*rry* (Figure 1) fused to complement receptor 2 (CR2). The CR2 component binds to the activation products iC3b/C3d/C3dg and thereby targets *Crry*-mediated complement inhibition specifically to sites of complement activation (13). In an effort to target complement activation/amplification downstream of the C3 convertase, the humanized anti-C5 monoclonal antibody (mAb) Eculizumab is being tested for efficacy in preventing post-transplant kidney DGF (NCT01403389; NCT01919346).

COMPLEMENT AND ALLOREACTIVE T CELLS

Building upon the paradigm-shifting observation that WT mice do not reject allografts from C3-deficient donors (27), work from several groups uncovered an unexpected role for immune cell-derived complement as a regulator of T cell immunity (Figure 2B). These studies showed that alternative pathway complement components are produced by T cells and APCs during cognate interactions (including but not limited to allo-reactions) (6, 28, 29). Locally produced C3a and C5a bind to their receptors expressed on the T cell and the APC, resulting in signals that induce T cell proliferation, inhibit T cell apoptosis and drive APC upregulation of costimulatory molecules and cytokines, together amplifying T cell immunity. C5a also has T cell chemoattractant properties via directly binding to C5aR on the T cells (30) and indirectly, through upregulating chemokines (17). Experiments performed in BM chimeric mice indicate that these effects are mediated through immune cell-derived, rather than systemic, complement (6, 31).

The effects of complement on alloreactive T cells impact transplant rejection. WT mice reject heart allografts deficient in the complement regulator DAF with accelerated kinetics through a process that is complement and T cell dependent (31). DAF physiologically restrains complement activation so its deficiency results in increased local production of C3a

and C5a which amplify alloreactive T cell responses, explaining the above observations (29, 32). DAF deficiency also accelerates T cell-dependent skin graft rejection (28) and murine graft versus host disease (GVHD) (33). Conversely, blocking C5a/C5aR interactions modulate T cell-dependent rodent kidney transplant rejection (34) and murine GVHD (33). Together with the observations that anti-C5 monoclonal antibody a) synergizes with CTLA4-Ig to prevent T cell priming, b) limits T cell trafficking to an allograft and c) prolongs transplant survival in mice (17), the body of work supports the conclusion that complement is a physiologically important regulator of pathogenic T cell immunity that causes allograft rejection in animal models.

Analogous mechanisms apply to human T cells. C3a and C5a are generated during *in vitro* cultures of human T cells responding to allogeneic DCs and mediate alloimmune T cell activation and expansion through the similar mechanisms delineated in the murine models (35, 36). Pharmacological C5aR blockade reduced human anti-mouse GVHD scores, and inhibited T cell responses in NOD/SCID/γc^{null} mouse recipients of human peripheral blood mononuclear cells (35). Consistent with a role for complement as a crucial mediator of human T cells, patients genetically deficient in C3 have impaired Th1 differentiation (37), and Compstatin, a C3 antagonist, was shown to inhibit human CD4 T cell proliferation and polarization (38). One unique difference between mice and humans delineated in 2013 is that human CD4+ T cells store intracellular C3. Upon T cell receptor (TCR) activation cathepsin L-mediated production of C3 cleavage leads to autocrine C3a-C3aR signaling that sustains T cell homeostasis and Th1/17 effector cell differentiation (39). Another publication from the same group showed that C3a/C3aR signaling synergizes with lipopolysaccharide (LPS)-transmitted signals on human monocytes to induce inflammasome activation, secretion of IL-1β and differentiation of IL-17-producing alloreactive T cells (40). Together, the findings substantiate a key role for complement in human effector T cell immunity.

Three 2013 publications showed that immune cell-derived complement inhibits induction, function and stability of regulatory T cells (Treg), including those required for induction/maintenance of allograft tolerance (41–44) (Figure 2B). Genetic or pharmacological blockade of C3aR/C5aR signal transduction in thymic-derived or natural Treg (nTreg) augments their *in vitro* and *in vivo* suppressive activity, abrogates autoimmune colitis, and prolongs allogeneic skin graft survival. Mechanisms involve C3a/C3aR and C5a/C5aR-induced phosphorylation of AKT in the Treg and, as a consequence, phosphorylation of the transcription factor Foxo1, which results in lowered nTreg Foxp3 expression. Genetic deficiency or pharmacological blockade of C3aR/C5aR signaling also augments generation of Treg from naive precursors (induced or iTreg), stabilizes Foxp3 expression in iTreg, resists iTreg conversion to IFNγ/TNFα-producing effector T cells (42, 43).

Immune cell-derived complement similarly modulates human Treg generation and function *in vitro* and *in vivo* (42). Building upon previously published evidence that coengagement of the TCR and the complement regulator CD46 promote regulatory IL-10 production (45), these new translational results underscore the crucial role of complement in modulating the balance between pathogenic and protective adaptive T cell responses. They provide proof-of-concept that C3a/C3aR and C5a/C5aR ligations are viable targets for inhibiting alloreactive Teff and facilitating iTreg-mediated transplant tolerance. Whether complement

antagonists can therapeutically control T cell alloreactivity while simultaneously promoting Treg-induction, function and stability to improve transplant outcomes in humans remains to be determined.

COMPLEMENT AND ANTIBODY-MEDIATED TRANSPLANT INJURY

It has been known for decades that complement regulates IgG production (46). The mechanism involves antigen-bound C3dg (a C3b cleavage product) binding to B cell-expressed CR2 (CD21), which facilitates antigen presentation to B cells, and lowers the threshold for B cell activation (47, 48). C3-deficient mice fail to produce high-affinity IgG responses against major histocompatibility antigens in skin grafts (49), confirming relevance to transplantation. Helper signals provided by T follicular helper (T_{FH}) cells to support the differentiation of antigen-specific B cells into IgG-producing memory and plasma cells (reviewed in (50)). Whether complement specifically impacts this process remains to be determined. Additional evidence for a link between complement and humoral immunity derives from a 2013 publication in which the authors demonstrated that complement-dependent IR injury amplifies development of humoral immune responses through a factor B-dependent mechanism (16).

Complement activation is an established effector mechanism of alloantibody-mediated graft injury in rodent transplant models (2, 51) (Figure 2C). Donor specific antibodies (DSA) initiate complement activation via the classical pathway. Subsequent anaphylatoxin production and MAC formation mediate inflammation and injury (52). Similar mechanisms apply to human transplant recipients with donor-specific anti-HLA antibodies (1, 52–54).

A 2013 publication performed in a humanized mouse model of vascular transplantation provided new mechanistic insight into how complement links alloantibodies to allograft injury (55). The investigators demonstrated that DSA bound to the donor human vascular endothelial cells, caused complement deposition, activation, and MAC formation, and led to non-canonical NF-κB signaling. The complement activation did not result in cell lysis but instead initiated a pro-inflammatory gene program that facilitated recruitment of alloreactive T cells required for the development of the allograft injury (Figure 2C).

Building upon the mechanistic studies, the availability of Eculizumab, a mAb that blocks the C5 convertase, led to clinical trials testing distal complement inhibition as a means to prevent and/or treat antibody-mediated rejection (ABMR) in humans (off label, not FDA approved). Eculizumb plus plasma exchange reduced the incidence of ABMR in 26 sensitized kidney transplant recipients compared to a historical control group treated with a plasma exchange-based protocol alone (56). Eculizumab also successfully reversed established antibody-mediated rejection in a small cohort (57) although not universally (58). Later observations that ABMR can be resistant to Eculizumab (56) suggest that complement-mediated inflammation upstream of C5 or non-complement mediated mechanisms (e.g. Fc receptor dependent) contribute to disease pathogenesis. Other reagents are being developed and tested as well. Among them, C1-INH partially inhibited ABMR in baboons (23) and a novel peptide inhibitor of C1 (PIC1) inhibited antibody-initiated classical pathway and lectin pathway complement activation in rodents (59, 60).

With the recognition that DSA initiates complement activation and injury via the classical pathway, investigators hypothesized that detection of serum anti-HLA antibodies capable of binding C1q would enhance the prognostic utility of serum alloantibody analysis in kidney transplantation (61, 62). The latest iteration of the single antigen bead technology currently used for detecting anti-HLA antibodies (63) additionally identifies those antibodies that bind C1q. A 2013 population-based study of 1016 kidney transplant recipients suggested that, amongst patients with anti-HLA antibodies, those that were $C1q^+$ had the worst graft survival (64). Similar associations were documented in heart (65) and in lung transplants (66) recipients. The clinical importance of C1q binding remains unclear as other reports suggest that C1q binding adds little prognostic information beyond standard DSA testing (67, 68)

COMPLEMENT AND CHRONIC ALLOGRAFT INJURY

Mechanisms of late graft failure are complex and involve immune and non-immune mechanisms (69), but late graft loss is routinely associated with pathological evidence of progressive glomerulosclerosis, tubular atrophy, interstitial fibrosis, and arteriosclerosis (70, 71). Evidence suggests that intra-graft complement activation contributes to this pathogenic process (Figure 2D). C3-deficient kidney isografts transplanted into WT recipients were protected from toxin-induced tubular damage, proteinuria and progressive renal failure, despite the presence of abundant circulating C3 (72). Follow-up work showed that C3 is implicated in the activation of the renin-angiotensin system and in the epithelial to mesenchymal transition (73, 74) supporting the concept that synthesis of complement components by renal epithelial cells is one critical mediator of tubular damage in proteinuria-associated renal disease. A 2013 publication suggested that cyclosporine A-induced microparticle release causes activation of alternative pathway complement in endothelial cells and kidney allograft injury (75). In a 2014 paper, the investigators showed that endothelial-to-mesenchymal transition and associated renal fibrosis are linked to C3a/C5a via AKT activation (76). Together, these results raise the possibility that kidney-derived complement participates in the development of kidney post-transplant IF/TA. Other murine studies indicate complement activation contributes to chronic allograft injury in other organs, including obliterative bronchiolitis following lung transplantation (77–79).

Associative evidence linking complement to progressive transplant injury in humans derives from studies of complement gene polymorphisms, serum complement concentrations and transplant outcomes in humans. Specific C5 polymorphisms in both the donor and the recipient have been associated with worse late graft function, but interestingly not with the risk of acute rejection (80). Although controversial, some additional evidence suggests that donor kidney expression of a specific polymorphic variant of C3 is associated with worse post-transplant outcomes (81, 82). Low C4 gene copy numbers (<4) in kidney transplant recipients were also associated with increased allograft survival (83). Additional evidence linking complement to chronic human allograft injury includes a proteomic analysis of kidney allograft tissue which showed strong associations between IF/TA and alternative pathway complement components (84). An ongoing study of chronic Eculizumab therapy in kidney transplant recipients (NCT01327573) could potentially provide further insight into the role of complement as a mediator of progressive graft dysfunction and IF/TA.

CONCLUSIONS

The complement system is now firmly established as a pervasive mediator of transplant injury in animal models, and evidence supports that these newly recognized mechanisms apply to human transplant recipients (Figure 2). This success of translational immunology along with the development of pharmacological agents that block human complement components and receptors (85, 86), will permit testing of the intriguing concept that targeting complement in all solid organ transplant recipients will improve graft survival and patient health.

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Key points

- The complement system negatively impacts the allograft survival via multiple mechanisms.
- Serum complement participates in antibody initiated allograft injury, locally produced, graft-derived, complement mediates IR injury, and immune cell-derived mediates crucially modulates effector and regulatory T cells
- Targeting complement has the potential to attenuate alloimmunity and allograft injury thereby improving outcomes in human transplant recipients

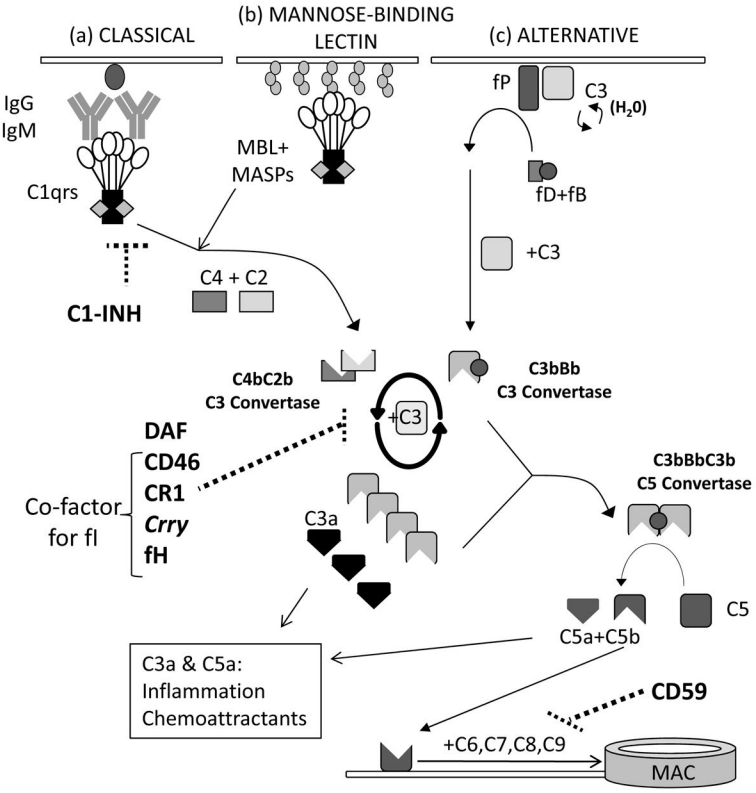

Figure 1.

Overview of the complement cascade and its regulators. Complement activation can be initiated by the classical pathway that is triggered by cross-linking, cell-bound subclasses of IgG and IgM antibodies (a), the mannose binding lectin (MBL) pathway triggered by carbohydrates present on bacteria surface (b), and the alternative pathway that undergoes spontaneous activation on cell surfaces (c). All three pathways converge into one key amplification step to form multimeric C3 convertases which cleave C3 to C3a and C3b, the latter initiating formation of the C5 convertase. Subsequently C5 cleavage yields C5a and C5b, ultimately forming the membrane attack complex (MAC, C5b-9) on the target cells. Complement activation/amplification is restrained on self-cells by several membrane-bound and soluble regulatory proteins. Surface-expressed regulators include Decay Accelerating Factor (DAF or CD55, accelerates the decay of cell-surface assembled C3 convertases), CD46 [membrane cofactor protein, MCP, co-factor for factor I (soluble) that inactivates C3b to iC3b], *Crry*, the murine homologue of CD46 that has both decay accelerating and co-factor activities, complement receptor 1, (CR1, CD35, binds to C3b/C4b and has co-factor activity) and CD59 (protectin, inhibits formation of the MAC). Factor H is a soluble complement regulator that exhibits both decay accelerating and co-factor activity. C1 inhibitor (C1-INH) disassociates the C1qrs complex (among other actions) limiting classical pathway activation. C1-INH: C1 inhibitor; CR1: complement receptor 1 (CD35); DAF: decay accelerating factor (CD55), fB: factor B; fD: factor D; fH: factor H; fI: factor I; fP: properdin; MAC: membrane attack complex C5b-9; MBL: mannose-binding lectin; MASP: mannan-binding serine peptidase.

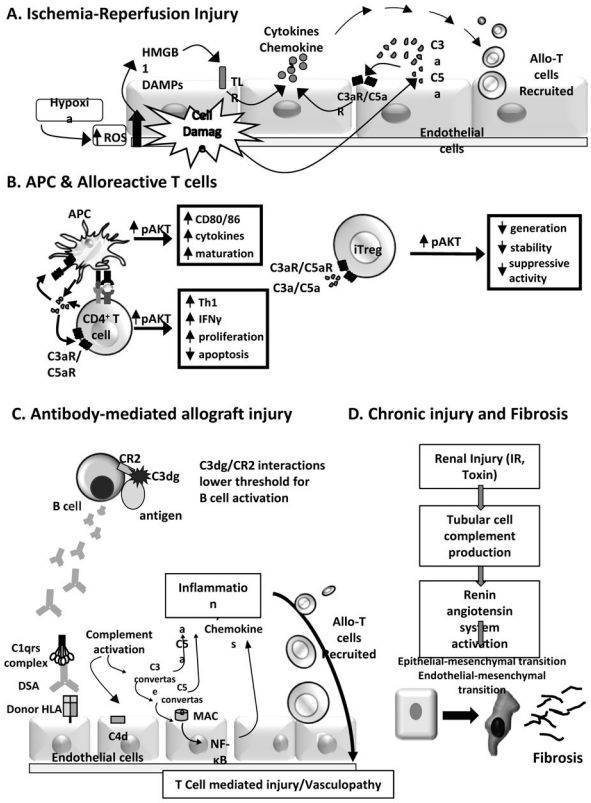

Figure 2. Mechanisms through which complement mediates transplant injury

A) Ischemia-Reperfusion Injury. Following reperfusion the generation of reactive oxygen species (ROS) is associated with graft-derived complement production and local activation as well as directly causing endothelial damage and release of damage-associated molecular patterns (DAMPs, e.g. HMGB1). Subsequent Toll-like receptor (TLR) signaling synergizes with and amplifies complement activation yeilding C3a and C5a. These anaphylatoxins signal through their receptors on endothelial cells (among other targets) inducing chemokine release and facilitating T cell infiltration into the allograft. B) APC & Alloreactive T cells. Cognate interactions between APCs and T cells yield immune cell complement production which activates through the alternative pathway to yield C3a and C5a. The anaphylatoxins bind to their receptors on both partners to induce APC maturation, and effector T cell proliferation/expansion, survival, and differentiation. The same signals inhibit generation, stability, and function of Treg. C) Antibody-mediated allograft injury. Alloantigen-bound C3dg (a C3b cleavage product) binds to B cell-expressed complement receptor 2 (CD21) to facilitate antigen presentation to lowers the threshold for B cell activation. Donor specific antibodies that bind to endothelial cells initiate the classical pathway activation, leading to complement deposition/activation, local C3a/C5a production and MAC formation. The latter results in non-canonical NF-κB signaling that initiates a proinflammatory gene program, enhancing recruitment of allreactive T cells and development of vasculopathy. D) Chronic graft injury and fibrosis. Intra-graft complement production/activation to activation of the renin-angiotensin system and epithelial-to-mesenchymal transition. C3a/C5a produced by endothelial cells induce endothelial-to-mesenchymal transition and renal firbrosis.