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Complement and Transplantation: From New Mechanisms to Potential Biomarkers and Novel Treatment Strategies

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INTRODUCTION

The complement system is traditionally considered a component of the innate immune system. In the context of transplantation, evidence published since the late 1990s has expanded our understanding of links between complement and adaptive immunity, including alloimmune responses and transplant injury. Among many pathogenic functions in transplantation, complement participates in ischemia-reperfusion (I/R) injury, controls the strength of the donor-reactive T cell immune response, and acts as an effector for antibody-initiated allograft injury. Herein, we will review the biology of the complement system and discuss current concepts regarding how complement contributes to the pathogenesis of transplant injury. We will also discuss how the discovery of these fundamental mechanisms has led to development and testing of a) novel treatment strategies that could limit allograft injury and improve the health of transplant recipients and b) complement-derived biomarkers for post-transplant risk stratification.

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BIOLOGY OF THE COMPLEMENT SYSTEM

The complement system is comprised of >30 soluble and membrane-bound proteins, including zymogens, receptors, and regulators. The individual complement components can be categorized by their ability to initiate, amplify, or regulate the complement cascade and/or perform effector functions (Figure 1).

Complement activation can be initiated via the classical pathway, the lectin pathway, and the alternative pathway ¹. The classical pathway is activated when C1q, as part of the C1qrs complex, binds to the Fc regions of IgG or IgM. The activated C1qrs complex then cleaves C4 and C2, forming the membrane bound C4bC2b C3 convertase, which enzymatically cleaves C3 into C3a (an anaphylatoxin) and C3b. In the lectin pathway, mannose-binding lectins (MBLs), as well as collectins and ficolins, function as pattern recognition receptors and bind bacterial carbohydrate and/or lipid motifs. MBL can also directly recognize neoantigens expressed by injured or apoptotic cells ², a mechanism pathogenically linked to ischemia reperfusion injury (see discussion below). Once bound, the lectin binding proteins function as opsonins and also interact with MBL-associated serine proteases (MASPs). Upon binding to MBL, MASP-1 and MASP-2 act analogously to the C1qrs complex; the MBL/MASP-1/2 complex that cleaves C4 and C2 to generate C4bC2b C3 convertases. In the alternative pathway, complement activation occurs continuously and spontaneously at low levels, through a process commonly referred to as “tickover.” This process is initiated by spontaneous hydrolysis of C3, forming C3_(H2O), which permits binding of Factor B (fB). Factor D (fD)-mediated cleavage of C3_(H2O) bound fB forms the initial C3 convertase, C3_(H2O)Bb, which cleaves C3 into C3a and C3b. C3b then associates with Bb to form the membrane bound C3bBb alternative pathway C3 convertase. Once C3 convertases are formed by any of the 3 activation pathways, the alternative pathway amplifies complement activation by utilizing the locally produced C3b molecules to form more C3 convertases.

The surface bound, multimeric, C3 convertases can bind additional C3b molecules to yield the C5 convertases, C4bC2bC3b and C3bBbC3b (Figure 1). These enzymes cleave C5 into C5a (another anaphylatoxin) and C5b, the latter initiating the formation of the C5b-9 membrane attack complex (MAC) ³. The MAC forms a pore in cell membranes, which promotes cytolysis in non-nucleated cells (including bacteria and red blood cells). MACs inserted into nucleated cells generally induce cellular activation ⁴, rather than lysis, and/or promote tissue injury ⁵. Soluble and surface bound split products, including C3a, C3b, iC3b, C3dg and C5a mediate separate but overlapping effector functions ⁶. C3a and C5a ligate their respective receptors C3aR1 and C5aR1, 7-transmembrane-spanning, G-protein coupled receptors (GPCR) expressed on various cell types to mediate chemotaxis, cytokine release, antigen-presenting cell (APC) activation, and T-cell activation and expansion (see below) ⁷⁻¹⁰. C3b, iC3b, and C3dg bind to various complement receptors, functioning as opsonins.

Under physiological conditions, multiple regulatory mechanisms throughout the cascade prevent injury to self-cells but permit complement activation on pathogens that do not express complement regulators ⁶ (Figure 1). The C1-inhibitor (C1-INH) inactivates the C1qrs complex, as well as the MBL/MASP-1/MASP-2 complex, to prevent classical and lectin pathway activation. Decay accelerating factor (DAF; CD55), a

glycophosphatidylinositol-(GPI)-anchored, cell surface expressed protein, accelerates the decay of the C3 convertases, thereby limiting amplification of the complement cascade. Soluble factor H and surface-expressed membrane cofactor protein (MCP, CD46) are cofactors for factor I that cleave C3b into iC3b, a mechanism that irreversibly disassociates C3 convertases. Downstream, ubiquitously expressed carboxypeptidases rapidly inactivate the C3a and C5a anaphylatoxins while the surface expressed, GPI-anchored protein CD59 (protectin) prevents the formation of MACs.

While the liver produces the majority of the circulating (plasma) complement components, complement proteins are locally produced by multiple other cell types including endothelial cells, parenchymal cells [*e.g.* tubular cells in the kidney ¹¹] and immune cells, including T cells and antigen presenting cells (APCs) ¹².

COMPLEMENT AND ISCHEMIA-REPERFUSION INJURY

Static cold storage of donor organs induces tissue hypoxia, mitochondrial damage, and ATP depletion, which, upon reperfusion, results in the generation of free oxygen radicals and organ damage commonly referred to as ischemia reperfusion (I/R) injury ¹³. In kidney transplantation, I/R injury presents clinically as delayed graft function (DGF). Extensive experimental evidence now supports a crucial role for complement in this process (see Figure 2). I/R-induced injury to endothelial cells results in surface expression of neoantigens that can be recognized by MBL ^{14–16} or collectins ¹⁷. Subsequent complement activation yields C3a and C5a that bind to their respective receptors on endothelial cells (among other cell types). These ligations crucially mediate I/R injury ^{18–20} (Figure 2). Data from animal models also suggests that donor brain death upregulates complement activation in renal allografts prior to procurement, which provides the dominant source of complement driving I/R injury ^{21,22}. The elucidation of these mechanisms has resulted in novel strategies aimed at preventing I/R injury and DGF. Chun et al showed in 2018 that peri-transplant treatment with C1-INH (to block MBL and classical pathway complement activation) overcame prolonged cold ischemia-induced, complement-dependent, I/R injury and cardiac allograft rejection in a mouse model ²³. Subsequent work by Jordan and colleagues translationally showed that C1-INH administration can limit DGF and improve kidney function in human recipients of deceased donor kidneys ²⁴ C1-INH also improved lung function in a small cohort of lung transplant patients with early, primary graft dysfunction (a form of I/R injury) ²⁵, further highlighting the utility of this treatment strategy.

In another approach targeting complement-dependent injury, investigators treated donor lungs with a nebulized C3a receptor (C3aR1) antagonist to ameliorate brain death-associated I/R injury in a murine lung transplant model ²⁶. Additionally, mirococept (APT070), a lipid-tailed C3 convertase inhibitor that can be perfused into donor organs and inserts into the endothelial cell membranes within the allograft has been shown to prevent kidney I/R injury in rodents ²⁷ and to improve early islet allograft inflammation in a humanized mouse model ²⁸. Studies testing efficacy of mirococept to prevent DGF in human recipients of deceased donor kidneys are ongoing ²⁹. In contrast to the positive effects of C1-INH alluded to above, a recent prospective, randomized trial testing anti-C5 mAb, eculizumab, in kidney

transplantation showed no effect in preventing DGF³⁰, suggesting that prevention of DGF requires proximal complement inhibition (at or prior to the C3 convertase step).

COMPLEMENT AND T CELL- MEDIATED REJECTION

Building upon the paradigm-shifting observation that C3-deficiency prolongs murine renal allograft survival³¹, work from several groups revealed an unanticipated role for immune cell-derived complement as a regulator of T cells, including T cells reactive to transplant antigens (Figure 3). These studies showed that during cognate interactions between CD4⁺ T cells and APCs, both cell types downregulate surface expression of DAF and secrete alternative pathway complement components which together result in local production of C3a and C5a. The interacting partners also upregulate surface expression C3aR1 and C5aR1 during activation³². Subsequent autocrine and paracrine C3a/C3aR1 and C5a/C5aR1 signaling via the phosphatidylinositol-3 kinase-gamma (PI3K γ) pathway induces alloreactive, effector T cell proliferation/differentiation and inhibits T cell apoptosis. Simultaneously, immune cell-derived C3a/C5a ligations with their receptors on APCs induce upregulation of costimulatory molecules and cytokines³² to further amplify the effector T cell response. Remarkably, Sheen et al showed in 2017 that Toll-like receptor-induced APC activation, a process thought to be crucial for protective immune responses against pathogens and for pathogenic immune responses directed at transplanted organs, requires immune cell production of complement and subsequent C3a/C3aR1 and C5a/C5aR1 ligations on the APC³³.

Analogous processes apply to human T cell/APC interactions. C3a and C5a are locally produced during *in vitro* cultures of human T cells and allogeneic dendritic cells (DCs), resulting in alloreactive T cell activation and expansion^{34,35}. Separate work by Kemper and colleagues showed a distinct role for complement in human T cell immunity by demonstrating that the intracellular production of complement cleavage products, including C5a, is crucially involved in human T cell activation, in part via activation of the inflammasome³⁶. Consistent with a role for complement as a modulator of human T cells, patients genetically deficient in C3 have impaired Th1 differentiation³⁷.

In contrast to complement-dependent promotion of effector T cell responses, immune cell-derived, locally produced C3a/C5a inhibits the generation and stability of regulatory T cells (Treg) in mice and humans^{9,10,38} (Figure 3), which contributes to augmenting the antigen-specific effector T cell response. The inhibitory effects of C3aR1/C5aR1 signaling on Treg generation are modulated by T cell expressed C5aR2 that binds C5a but lacks the intracellular signaling domain to initiate G-protein coupled receptor signaling³⁹. Evidence suggests that in T cells, C5aR2 functions to bind C5a and prevent C5a from binding to and activating C5aR1. Verghese et al showed in 2018 that absence of C5aR2 limits, while transgenic overexpression of C5aR2 enhances, *in vivo* Treg generation, independent of C5aR1 expression on the T cells⁴⁰

Mechanistic experiments showed that C3aR1/C5aR1-initiated signaling, which is prevented by overexpression of C5aR2³, results in AKT-dependent phosphorylation of the transcription factor Foxo1, preventing its nuclear translocation and subsequently limiting

FoxP3 transcription¹⁰. Conversely, disrupting C3aR1/C5aR1 signaling on T cells genetically or pharmacologically^{10,38} augments Treg generation, stabilizes FoxP3 expression and is protolerogenic.

Complement regulation of T cell immunity is broadly relevant and applies in the context of infectious pathogens⁴¹, autoimmune diseases⁴², and T cell driven transplant rejection. As examples of the latter, absence of DAF (lifts restraint on production of C3a and C5a⁴³) accelerates cardiac allograft rejection⁴⁴ while anti-C5 mAb⁴⁵, C5aR1 antagonism⁴⁶, or absence of C3aR1 signaling⁴⁷ reduces T cell priming and improves allograft survival in mice. Similarly, Treg-dependent allograft survival/tolerance is facilitated by blocking C3aR1/C5aR1 signaling⁹ or augmenting C5aR2 expression on immune cells⁴⁰. Together with the observations that these mechanisms apply to human T cells³⁴, the data support the need to test the clinical impact of complement inhibition on T-cell-mediated allograft rejection in humans.

COMPLEMENT AND ANTIBODY-MEDIATED TRANSPLANT INJURY

Complement has established effects on antibody production and function. Complement receptor 1 (CR1, CD35) and complement receptor 2 (CR2, CD21) bind to antigen-bound C3b and iC3b, respectively, the latter lowering the threshold for B cell activation and promoting antigen retention by follicular dendritic cells^{48,49}. C3-deficient mice fail to produce high-affinity IgG responses against major histocompatibility antigens in skin grafts⁵⁰, confirming its relevance to transplantation.

In addition to complement's role in donor specific antibody (DSA) production, complement activation has been shown to be an important effector mechanism of antibody-initiated allograft injury in murine models⁵¹ and human transplant recipients^{52,53} (Figure 4). DSA can activate complement via the classical pathway, ultimately inducing graft inflammation and injury through C3a/C5a production and MAC formation⁵². While MAC formation mediates lysis in non-nucleated cells, terminal complement activation on donor human endothelial cells induces non-canonical NF- κ B signaling, initiating a pro-inflammatory gene program that facilitates recruitment of alloreactive T cells required for the development of allograft injury⁵⁴

Building upon these fundamental observations, clinicians have studied the efficacy of eculizumab, an anti-human C5 mAb, to prevent and/or treat antibody-mediated rejection (ABMR) in human transplant recipients. Eculizumab plus plasma exchange reduced the incidence of ABMR in 26 sensitized kidney transplant recipients compared to a control group⁵⁵, and it successfully treated ABMR in a small cohort⁵⁶, although it failed to demonstrate efficacy in another case series⁵⁷. Notably, ABMR can be resistant to eculizumab⁵⁵, suggesting that complement components proximal to the C5 convertase and/or non-complement-(e.g. Fc receptor)-dependent effector mechanisms can contribute to the antibody-initiated pathology. A pilot study of C1-INH, which blocks activation of the classical and MBL pathways (Figure 1), suggests efficacy for treatment of ABMR⁵⁸, but larger scale studies are needed.

COMPLEMENT AND TRANSPLANT REJECTION BIOMARKERS

With the recognition that complement activation is a critical mediator of I/R injury, T-cell mediated rejection, and ABMR, investigators have begun to test whether complement-derived biomarkers could be useful as prognostic risk-assessment tools in transplantation. To this end, research on potential biomarkers has focused on three broad areas: single nucleotide polymorphisms (SNPs) in complement genes, measures of complement-binding capacity of DSA, and detection of serum-based complement components.

Both functional and non-functional SNPs have been identified in complement components throughout the cascade, including within the complement regulators. Their functional effects and relevance to renal transplant outcomes are summarized in a 2017 review⁵⁹. Recipient SNPs in C5 (rs17611, increases serum C5a levels) and C5aR1 (rs4804049, nonfunctional) have been linked to worse renal allograft function at 1 year post-transplant and allograft failure, respectively. Similarly, two C3 SNPs (rs10411506 and rs2230205) correlate with ABMR in renal transplant recipients⁶⁰. As donor-expressed complement regulatory proteins could confer protection from DSA⁶¹, investigators have hypothesized that donor SNPs within regulators could also be used to assess post-transplant risk. Indeed, a donor CD59 promoter polymorphism (rs147788946) is associated with an increased rate of early acute rejection in renal allografts⁶² and a higher incidence of chronic rejection among lung transplant recipients⁶³. Going forward, sufficiently powered validation studies will be crucial in ultimately determining whether treatment strategies should be individualized based on expression of the various SNPs.

DSA-associated allograft injury is in part dependent on complement-mediated effector functions, so the prognostic value of the complement-binding capacity of anti-HLA antibodies has become an important question in transplantation. A 2013 cohort study of 1016 kidney transplant recipients suggested that patients with C1q-binding (C1q⁺) DSA have elevated rates of ABMR compared to C1q⁻ DSA⁺ patients (48% vs 16%)⁶⁴. Similar associations were documented in heart⁶⁵ and in lung⁶⁶ transplant recipients. In another cohort study of 70 renal transplant patients that developed de novo DSA (dnDSA), C1q-binding was correlated with post-dnDSA graft loss⁶⁷. However, the authors of this study also demonstrated that C1q positivity correlated with DSA titer and mean fluorescence intensity (MFI), suggesting that C1q binding may simply reflect the strength of the serum DSA and may not add significant prognostic information beyond standard DSA testing.

A non-invasive, serum-based biomarker for allograft rejection could help avoid unnecessary biopsies and lead to earlier treatment, potentially improving transplant outcomes. While no such validated biomarker currently exists in transplantation, there are complement-related candidates. Soluble CD59 (sCD59), which is associated with cellular damage, including post-myocardial infarction⁶⁸, was found to be elevated in a cohort of patients who developed bronchiolitis obliterans syndrome (BOS) after lung transplantation⁶⁹. Importantly, serum sCD59 concentrations were elevated prior to the clinical diagnosis of BOS in these patients, and sCD59 was determined to be an independent predictor for the development of BOS. In another study, investigators demonstrated a correlation between serum C4d⁺ (a marker of classical pathway complement activation) microvesicles and

ABMR in a cohort of kidney transplant patients⁷⁰; treatment of their ABMR was associated with an improvement in estimated glomerular filtration rate and a reduction in C4d⁺ microvesicle concentration. With regard to acute cellular rejection (ACR), a 2018 study found an association between circulating immune complexes, specific for complement factor H, and ACR in liver transplant recipients⁷¹. These intriguing findings support the need for further testing of complement biomarkers and/or complement biomarker profiles aimed at individualizing risk stratification and treatment algorithms for transplant patients.

CONCLUDING REMARKS

The complement system, traditionally considered a component of innate immunity, is now recognized as a crucial mediator of the adaptive immune response in solid organ transplantation. Pre-clinical and early human trials have begun to elucidate how specific complement components modulate the various forms of post-transplant anti-graft immune responses (e.g. I/R injury, T-cell mediated cellular rejection, ABMR). These data, together with the pharmaceutical industry's increasing interest in developing complement inhibitors⁷², support the need to test whether complement inhibition, potentially guided by complement-derived risk assessing biomarkers, can improve allograft survival and patient-centered outcomes in transplantation.

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REFERENCES

- Walport MJ. Complement. First of two parts. *N Engl J Med*. 2001;344(14):1058–1066. [PubMed: 11287977]
- Takahashi K, Ip WE, Michelow IC, Ezekowitz RA. The mannose-binding lectin: a prototypic pattern recognition molecule. *Curr Opin Immunol*. 2006;18(1): 16–23. [PubMed: 16368230]
- Bamberg CE, Mackay CR, Lee H, et al. The C5a receptor (C5aR) C5L2 is a modulator of C5aR-mediated signal transduction. *J Biol Chem*. 2010;285(10):7633–7644. [PubMed: 20044484]
- Park P, Haas M, Cunningham PN, et al. Inhibiting the complement system does not reduce injury in renal ischemia reperfusion. *J Am Soc Nephrol*. 2001;12(7):1383–1390. [PubMed: 11423567]
- Adler S, Baker PJ, Johnson RJ, Ochi RF, Pritzl P, Couser WG. Complement membrane attack complex stimulates production of reactive oxygen metabolites by cultured rat mesangial cells. *J Clin Invest*. 1986;77(3):762–767. [PubMed: 3005365]
- Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. 2010;11(9):785–797. [PubMed: 20720586]
- Guo RF, Ward PA. Role of C5a in inflammatory responses. *Annu Rev Immunol*. 2005;23:821–852. [PubMed: 15771587]
- Klos A, Tenner AJ, Johswich KO, Ager RR, Reis ES, Kohl J. The role of the anaphylatoxins in health and disease. *Mol Immunol*. 2009;46(14):2753–2766. [PubMed: 19477527]
- Kwan WH, van der Touw W, Paz-Artal E, Li MO, Heeger PS. Signaling through C5a receptor and C3a receptor diminishes function of murine natural regulatory T cells. *J Exp Med*. 2013;210(2): 257–268. [PubMed: 23382542]
- van der Touw W, Cravedi P, Kwan WH, Paz-Artal E, Merad M, Heeger PS. Cutting edge: Receptors for C3a and C5a modulate stability of alloantigen-reactive induced regulatory T cells. *J Immunol*. 2013;190(12):5921–5925. [PubMed: 23690475]

11. Peake PW, O'Grady S, Pussell BA, Charlesworth JA. C3a is made by proximal tubular HK-2 cells and activates them via the C3a receptor. *Kidney Int.* 1999;56(5):1729–1736. [PubMed: 10571781]
12. Lalli PN, Strainic MG, Yang M, Lin F, Medof ME, Heeger PS. Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. *Blood.* 2008;112(5):1759–1766. [PubMed: 18567839]
13. Siedlecki A, Irish W, Brennan DC. Delayed graft function in the kidney transplant. *Am J Transplant.* 2011;11(11):2279–2296. [PubMed: 21929642]
14. Zhang M, Carroll MC. Natural IgM-mediated innate autoimmunity: a new target for early intervention of ischemia-reperfusion injury. *Expert Opin Biol Ther.* 2007;7(10):1575–1582. [PubMed: 17916049]
15. Haas MS, Alicot EM, Schuerpf F, et al. Blockade of self-reactive IgM significantly reduces injury in a murine model of acute myocardial infarction. *Cardiovasc Res.* 2010;87(4):618–627. [PubMed: 20462867]
16. Busche MN, Pavlov V, Takahashi K, Stahl GL. Myocardial ischemia and reperfusion injury is dependent on both IgM and mannose-binding lectin. *Am J Physiol Heart Circ Physiol.* 2009;297(5):H1853–1859. [PubMed: 19749170]
17. Nauser CL, Farrar CA, Sacks SH. Complement Recognition Pathways in Renal Transplantation. *J Am Soc Nephrol.* 2017;28(9):2571–2578. [PubMed: 28663231]
18. Thurman JM, Ljubanovic D, Edelstein CL, Gilkeson GS, Holers VM. Lack of a functional alternative complement pathway ameliorates ischemic acute renal failure in mice. *J Immunol.* 2003;170(3):1517–1523. [PubMed: 12538716]
19. De Vries B, Matthijsen RA, Wolfs TG, Van Bijnen AA, Heeringa P, Buurman WA. Inhibition of complement factor C5 protects against renal ischemia-reperfusion injury: inhibition of late apoptosis and inflammation. *Transplantation.* 2003;75(3):375–382. [PubMed: 12589162]
20. Peng Q, Li K, Smyth LA, et al. C3a and C5a promote renal ischemia-reperfusion injury. *J Am Soc Nephrol.* 2012;23(9):1474–1485. [PubMed: 22797180]
21. Farrar CA, Zhou W, Lin T, Sacks SH. Local extravascular pool of C3 is a determinant of postischemic acute renal failure. *FASEB J.* 2006;20(2):217–226. [PubMed: 16449793]
22. Atkinson C, Floerchinger B, Qiao F, et al. Donor brain death exacerbates complement-dependent ischemia/reperfusion injury in transplanted hearts. *Circulation.* 2013;127(12):1290–1299. [PubMed: 23443736]
23. Chun N, Fairchild RL, Li Y, et al. Complement Dependence of Murine Costimulatory Blockade-Resistant Cellular Cardiac Allograft Rejection. *Am J Transplant.* 2017; 17(11):2810–2819. [PubMed: 28444847]
24. Jordan SC, Choi J, Aubert O, et al. A phase I/II, double-blind, placebo-controlled study assessing safety and efficacy of C1 esterase inhibitor for prevention of delayed graft function in deceased donor kidney transplant recipients. *Am J Transplant.* 2018.
25. Sommer W, Tudorache I, Kuhn C, et al. C1-esterase-inhibitor for primary graft dysfunction in lung transplantation. *Transplantation.* 2014;97(11):1185–1191. [PubMed: 24573112]
26. Cheng Q, Patel K, Lei B, et al. Donor pretreatment with nebulized complement C3a receptor antagonist mitigates brain-death induced immunological injury post-lung transplant. *Am J Transplant.* 2018.
27. Patel H, Smith RA, Sacks SH, Zhou W. Therapeutic strategy with a membrane-localizing complement regulator to increase the number of usable donor organs after prolonged cold storage. *J Am Soc Nephrol.* 2006; 17(4): 1102–1111. [PubMed: 16510763]
28. Xiao F, Ma L, Zhao M, et al. APT070 (mirococept), a membrane-localizing C3 convertase inhibitor, attenuates early human islet allograft damage in vitro and in vivo in a humanized mouse model. *Br J Pharmacol.* 2016;173(3):575–587. [PubMed: 26565566]
29. Kassimatis T, Qasem A, Douiri A, et al. A double-blind randomised controlled investigation into the efficacy of Mirococept (APT070) for preventing ischaemia reperfusion injury in the kidney allograft (EMPIRIKAL): study protocol for a randomised controlled trial. *Trials.* 2017;18(1):255. [PubMed: 28587616]

30. Heeger P, Akalin E, Baweja M, et al. Lack of Efficacy of Eculizumab for Prevention of Delayed Graft Function (DGF) in Deceased Donor Kidney Transplant Recipients. *Am J Transplant.* 2018;18(S4).
31. Pratt JR, Basheer SA, Sacks SH. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat Med.* 2002;8(6):582–587. [PubMed: 12042808]
32. Strainic MG, Liu J, Huang D, et al. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4⁺ T cells. *Immunity.* 2008;28(3):425–435. [PubMed: 18328742]
33. Sheen JH, Strainic MG, Liu J, et al. TLR-Induced Murine Dendritic Cell (DC) Activation Requires DC-Intrinsic Complement. *J Immunol.* 2017;199(1):278–291. [PubMed: 28539427]
34. Cravedi P, Leventhal J, Lakhani P, Ward SC, Donovan MJ, Heeger PS. Immune cell-derived C3a and C5a costimulate human T cell alloimmunity. *Am J Transplant.* 2013;13(10):2530–2539. [PubMed: 24033923]
35. Li K, Fazekasova H, Wang N, et al. Expression of complement components, receptors and regulators by human dendritic cells. *Mol Immunol.* 2011 ;48(9–10):1121–1127. [PubMed: 21397947]
36. Liszewski MK, Kolev M, Le Friec G, et al. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity.* 2013;39(6):1143–1157. [PubMed: 24315997]
37. Ghannam A, Fauquert JL, Thomas C, Kemper C, Drouet C. Human complement C3 deficiency: Th1 induction requires T cell-derived complement C3a and CD46 activation. *Mol Immunol.* 2014;58(1):98–107. [PubMed: 24321396]
38. Strainic MG, Shevach EM, An F, Lin F, Medof ME. Absence of signaling into CD4(+) cells via C3aR and C5aR enables autoinductive TGF-beta1 signaling and induction of Foxp3(+) regulatory T cells. *Nat Immunol.* 2013;14(2):162–171. [PubMed: 23263555]
39. Okinaga S, Slattery D, Humbles A, et al. C5L2, a non-signaling C5A binding protein. *Biochemistry.* 2003;42(31):9406–9415. [PubMed: 12899627]
40. D AV, Demir M, Chun N, et al. T Cell Expression of C5a Receptor 2 Augments Murine Regulatory T Cell (TREG) Generation and TREG-Dependent Cardiac Allograft Survival. *J Immunol.* 2018;200(6):2186–2198. [PubMed: 29436411]
41. Stoermer KA, Morrison TE. Complement and viral pathogenesis. *Virology.* 2011 ;411 (2):362–373. [PubMed: 21292294]
42. Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: pathophysiological mechanisms. *J Immunol.* 2013;190(8):3831–3838. [PubMed: 23564577]
43. Lublin DM, Atkinson JP. Decay-accelerating factor: biochemistry, molecular biology, and function. *Annu Rev Immunol.* 1989;7:35–58. [PubMed: 2469439]
44. Pavlov V, Raedler H, Yuan S, et al. Donor deficiency of decay-accelerating factor accelerates murine T cell-mediated cardiac allograft rejection. *J Immunol.* 2008;181 (7):4580–4589. [PubMed: 18802060]
45. Raedler H, Vieyra MB, Leisman S, et al. Anti-complement component C5 mAb synergizes with CTLA4Ig to inhibit alloreactive T cells and prolong cardiac allograft survival in mice. *Am J Transplant.* 2011;11 (7): 1397–1406. [PubMed: 21668627]
46. Gueler F, Rong S, Gwinner W, et al. Complement 5a receptor inhibition improves renal allograft survival. *J Am Soc Nephrol.* 2008;19(12):2302–2312. [PubMed: 18753257]
47. Horwitz J, Mathern D, Heeger P. C3a Receptor Regulates the CD8 T-Cell Alloresponse via Intrinsic and Extrinsic Mechanisms *Am J Transplant.* 2018;18(S4).
48. Fang Y, Xu C, Fu YX, Holers VM, Molina H. Expression of complement receptors 1 and 2 on follicular dendritic cells is necessary for the generation of a strong antigen-specific IgG response. *J Immunol.* 1998;160(11):5273–5279. [PubMed: 9605124]
49. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science.* 1996;271 (5247):348–350. [PubMed: 8553069]

50. 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(7):1310–1318. [PubMed: 11602861]
51. Wang H, Arp J, Liu W, et al. Inhibition of terminal complement components in presensitized transplant recipients prevents antibody-mediated rejection leading to longterm graft survival and accommodation. *J Immunol*. 2007;179(7):4451–4463. [PubMed: 17878341]
52. Valenzuela NM, McNamara JT, Reed EF. Antibody-mediated graft injury: complement-dependent and complement-independent mechanisms. *Curr Opin Organ Transplant*. 2014;19(1):33–40. [PubMed: 24316758]
53. Stegall MD, Chedid MF, Cornell LD. The role of complement in antibody-mediated rejection in kidney transplantation. *Nat Rev Nephrol*. 2012;8(11):670–678. [PubMed: 23026942]
54. Jane-Wit D, Manes TD, Yi T, et al. Alloantibody and complement promote T cell-mediated cardiac allograft vasculopathy through noncanonical nuclear factor-kappaB signaling in endothelial cells. *Circulation*. 2013;128(23):2504–2516. [PubMed: 24045046]
55. Stegall MD, Diwan T, Raghavaiah S, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant*. 2011; 11(11):2405–2413. [PubMed: 21942930]
56. Locke JE, Magro CM, Singer AL, et al. The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am J Transplant*. 2009;9(1):231–235. [PubMed: 18976298]
57. Burbach M, Suberbielle C, Brocheriou I, et al. Report of the inefficacy of eculizumab in two cases of severe antibody-mediated rejection of renal grafts. *Transplantation*. 2014;98(10):1056–1059. [PubMed: 24839895]
58. Montgomery RA, Orandi BJ, Racusen L, et al. Plasma-Derived C1 Esterase Inhibitor for Acute Antibody-Mediated Rejection Following Kidney Transplantation: Results of a Randomized Double-Blind Placebo-Controlled Pilot Study. *Am J Transplant*. 2016;16(12):3468–3478. [PubMed: 27184779]
59. Michielsen LA, van Zuilen AD, Muskens IS, Verhaar MC, Otten HG. Complement Polymorphisms in Kidney Transplantation: Critical in Graft Rejection? *Am J Transplant*. 2017;17(8):2000–2007. [PubMed: 28097805]
60. Wang Z, Yang H, Guo M, et al. Impact of complement component 3/4/5 single nucleotide polymorphisms on renal transplant recipients with antibody-mediated rejection. *Oncotarget*. 2017;8(55):94539–94553. [PubMed: 29212248]
61. Griesemer AD, Okumi M, Shimizu A, et al. Upregulation of CD59: potential mechanism of accommodation in a large animal model. *Transplantation*. 2009;87(9):1308–1317. [PubMed: 19424030]
62. Michielsen LA, van Zuilen AD, Kardol-Hoefnagel T, Verhaar MC, Otten HG. Association Between Promoter Polymorphisms in CD46 and CD59 in Kidney Donors and Transplant Outcome. *Front Immunol*. 2018;9:972. [PubMed: 29867953]
63. Budding K, van de Graaf EA, Kardol-Hoefnagel T, et al. A Promoter Polymorphism in the CD59 Complement Regulatory Protein Gene in Donor Lungs Correlates With a Higher Risk for Chronic Rejection After Lung Transplantation. *Am J Transplant*. 2016;16(3):987–998. [PubMed: 26517734]
64. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med*. 2013;369(13):1215–1226. [PubMed: 24066742]
65. Zeevi A, Lunz J, Feingold B, et al. Persistent strong anti-HLA antibody at high titer is complement binding and associated with increased risk of antibody-mediated rejection in heart transplant recipients. *J Heart Lung Transplant*. 2013;32(1):98–105. [PubMed: 23142561]
66. Witt CA, Gaut JP, Yusen RD, et al. Acute antibody-mediated rejection after lung transplantation. *J Heart Lung Transplant*. 2013;32(10):1034–1040. [PubMed: 23953920]
67. Wiebe C, Gareau AJ, Pochinco D, et al. Evaluation of C1q Status and Titer of De Novo Donor-Specific Antibodies as Predictors of Allograft Survival. *Am J Transplant*. 2017;17(3):703–711. [PubMed: 27539748]

68. Vakeva A, Lehto T, Takala A, Meri S. Detection of a soluble form of the complement membrane attack complex inhibitor CD59 in plasma after acute myocardial infarction. *Scand J Immunol.* 2000;52(4):411–414. [PubMed: 11013013]
69. Budding K, van de Graaf EA, Kardol-Hoefnagel T, et al. Soluble CD59 is a Novel Biomarker for the Prediction of Obstructive Chronic Lung Allograft Dysfunction After Lung Transplantation. *Sci Rep.* 2016;6:26274. [PubMed: 27215188]
70. Tower CM, Reyes M, Nelson K, et al. Plasma C4d⁺ Endothelial Microvesicles Increase in Acute Antibody-Mediated Rejection. *Transplantation.* 2017;101(9):2235–2243. [PubMed: 27846156]
71. Aibara N, Ohyama K, Hidaka M, et al. Immune complexome analysis of antigens in circulating immune complexes from patients with acute cellular rejection after living donor liver transplantation. *Transpl Immunol.* 2018;48:60–64. [PubMed: 29477751]
72. Thurman JM, Le Quintrec M. Targeting the complement cascade: novel treatments coming down the pike. *Kidney Int.* 2016;90(4):746–752. [PubMed: 27325183]

KEY POINTS

- Complement activation, via the lectin and classical pathways, is an important mediator of ischemia-reperfusion injury.
- Local production of complement by immune cells induces antigen-presenting cell maturation and effector T cell expansion, while inhibiting regulatory T cell generation.
- Donor specific antibody (DSA)-mediated complement activation initiates a pro-inflammatory gene program in donor endothelial cells.
- Serum-based complement components have the potential to serve as biomarkers for allograft rejection.

SYNOPSIS

The complement system, traditionally considered a component of innate immunity, is now recognized as a crucial mediator of the adaptive immune response in solid organ transplantation. Pre-clinical and early human trials have demonstrated the importance of complement effector mechanisms in driving allograft injury during specific anti-graft immune responses, including ischemia-reperfusion injury, T cell-mediated rejection, and antibody-mediated rejection, as well as a potential role for complement-derived risk stratification biomarkers. These data support the need for further testing of complement inhibitors in solid organ transplant recipients.

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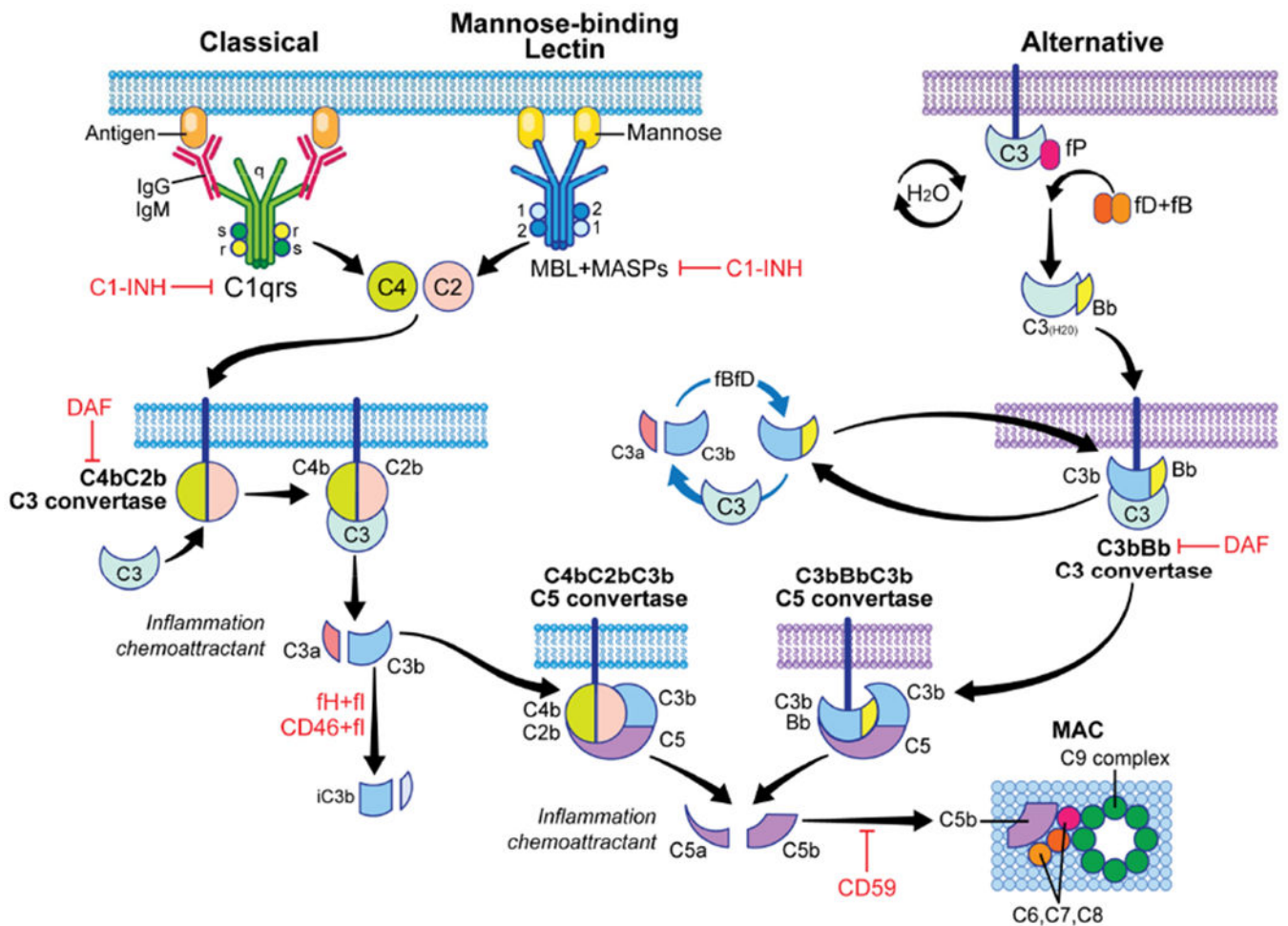


Figure 1. Overview of the complement cascade and its regulators.

Complement activation can be initiated by the classical pathway triggered by cross-linking cell-bound subclasses of IgG and IgM antibodies, the mannose binding lectin (MBL) pathway triggered by carbohydrates present on bacteria surface, and the alternative pathway that undergoes spontaneous activation on cell surfaces. All three pathways converge into one key amplification step to form multimeric C3 convertases which cleave C3 to C3a and C3b, the latter forming additional C3 convertases (amplification) and then 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 (shown in red). See text for further details. Surface-expressed regulators include decay accelerating factor (DAF or CD55, accelerates the decay of cell-surface assembled C3 convertases), membrane cofactor protein [MCP or CD46, co-factor for factor I (fI) that inactivates C3b to iC3b], and CD59 (protectin, inhibits formation of the MAC). Factor H (fH) is a soluble complement regulator that exhibits both decay accelerating and co-factor activity. C1 inhibitor (C1-INH) inhibits C1qrs and MBL-MASP complexes, limiting classical pathway and MBL pathway activation, respectively. MASP: mannose-associated serine protease.

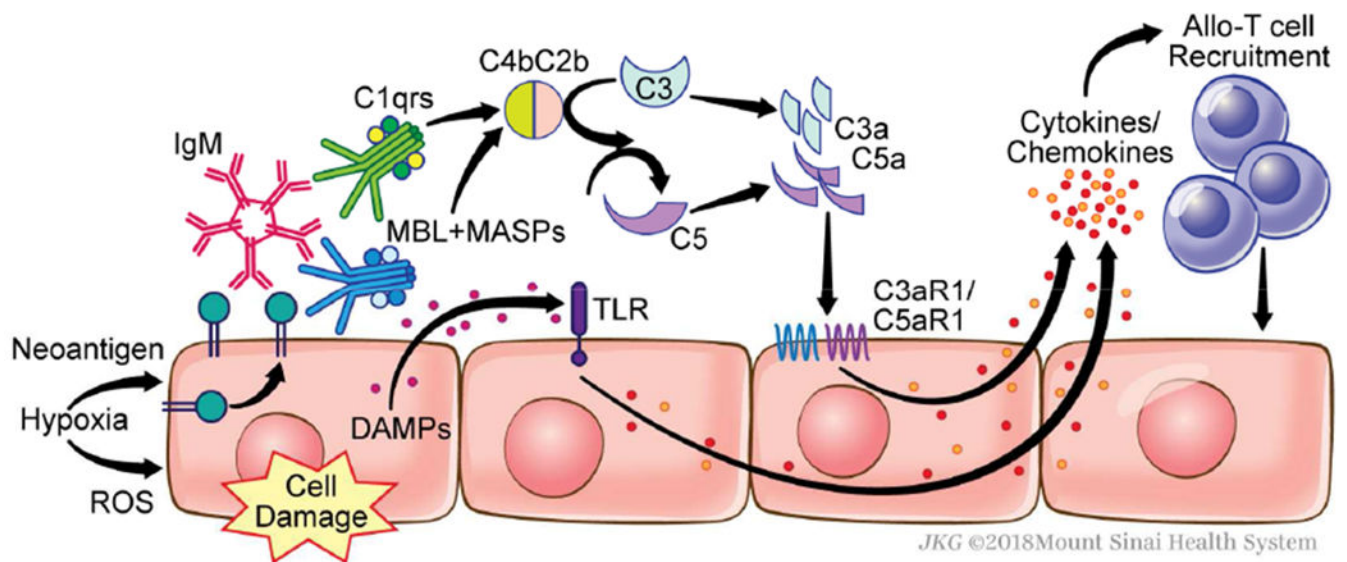


Figure 2. Mechanisms through which complement mediates ischemia-reperfusion injury. Hypoxia induces surface expression of neoantigens that are recognized by natural, pre-formed IgM and MBL (and/or collectins), which then initiate complement activation. Following reperfusion, the generation of reactive oxygen species (ROS) is associated with graft-derived complement production and activation as well as the release of damage-associated molecular patterns (DAMPs). Subsequent Toll-like receptor (TLR) signaling synergizes with and amplifies complement activation, together yielding C3a and C5a. These anaphylatoxins signal through their 7-transmembrane spanning G-protein coupled receptors on endothelial cells (among other cellular targets) inducing cytokine/chemokine release and facilitating T cell infiltration into the allograft.

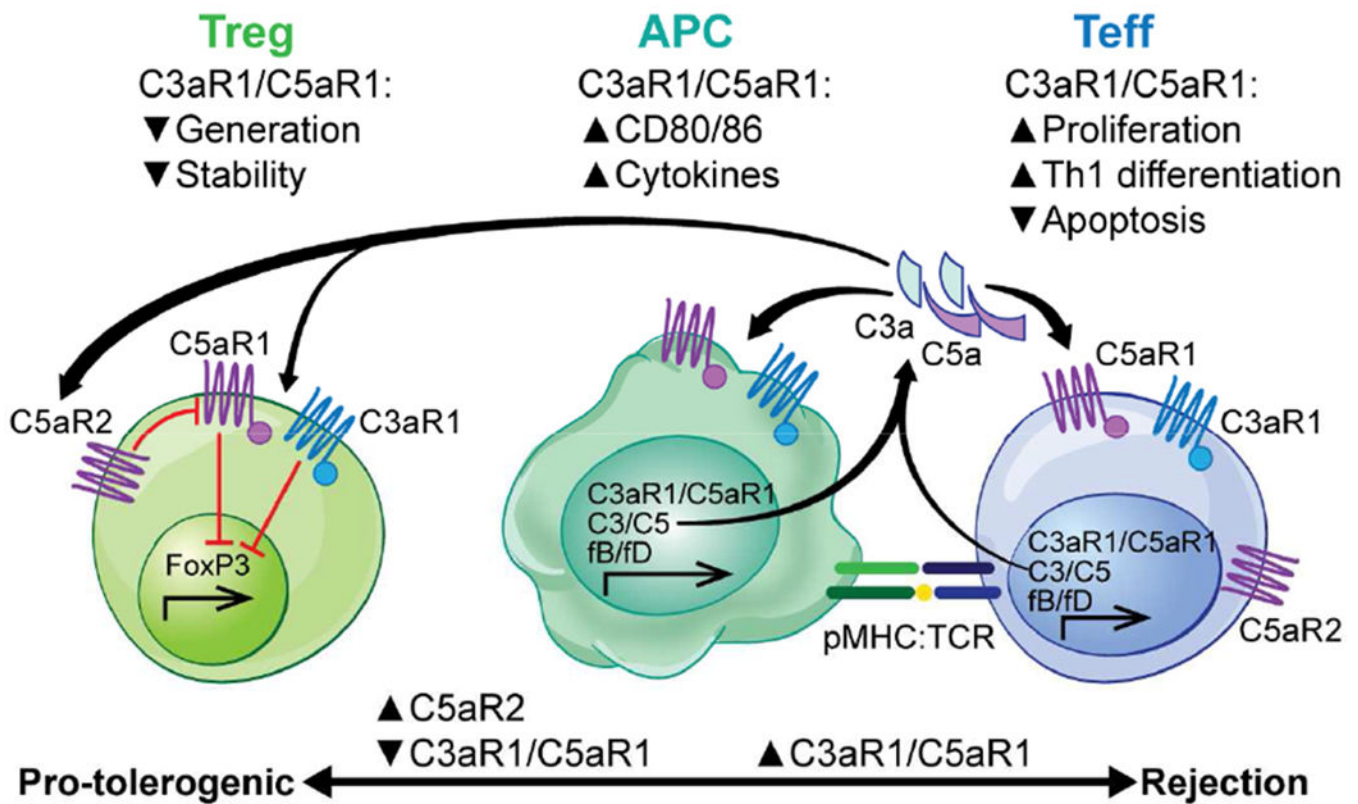


Figure 3. Mechanisms through which complement modulates T cell-mediated rejection. Cognate interactions between antigen presenting cells (APC) and T cells yield immune cell-derived complement production, which activates through the alternative pathway to yield C3a and C5a. The anaphylatoxins bind to their respective receptors (C3aR1 and C5aR1 respectively) on the interacting partners to induce APC maturation and effector T cell proliferation/expansion, survival, and differentiation. The same signals simultaneously inhibit the generation, stability, and function of regulatory T cells (Treg) in part by inhibiting Foxp3 expression. As C5aR2 binds C5a but lacks a GPCR signaling motif, C5aR2 expression on T cells limits the ability of free C5a to bind to C5aR1 and thus facilitates Treg induction by limiting C5a/C5aR1 ligations.

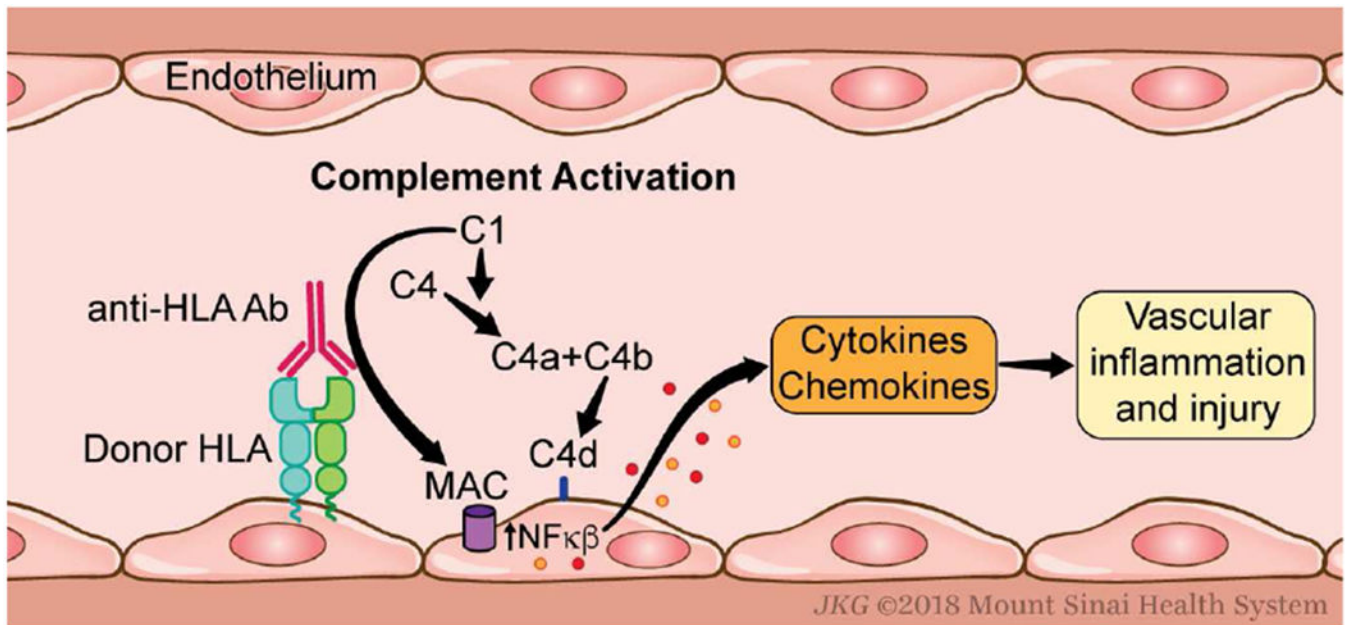


Figure 4. Mechanisms through which complement modulates antibody-mediated rejection. Donor specific antibodies (DSA) bind to donor human leukocyte antigen (HLA) on endothelial cells and initiate complement activation via the classical pathway, leading to complement deposition, local C3a/C5a production, and MAC formation. The latter causes non-canonical NF- κ B signaling that initiates a pro-inflammatory gene program, enhancing recruitment of alloreactive T cells and development of vascular injury.