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Complement Regulation of T-Cell Alloimmunity

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Summary

Complement proteins are generated both by the liver (systemic compartment) and by peripheral tissue-resident cells and migratory immune cells (local compartment). The immune cell-derived, alternative pathway complement components activate spontaneously, yielding local, but not systemic, production of C3a and C5a. These anaphylatoxins bind to their respective G-protein-coupled receptors, the C3a receptor and the C5a receptor, expressed on T cells and antigen-presenting cells, leading to their reciprocal activation and driving T-cell differentiation, expansion, and survival. Complement deficiency or blockade attenuates T-cell-mediated autoimmunity and delays allograft rejection in mice. Increasing complement activation, achieved by genetic removal of the complement regulatory protein decay accelerating factor, enhances murine T-cell immunity and accelerates allograft rejection. Signaling through the C3a receptor and the C5a receptor reduces suppressive activity of natural regulatory T cells and the generation and stability of induced regulatory T cells. The concepts, initially generated in mice, recently were confirmed in human immune cells, supporting the need for testing of complement targeting therapies in organ transplants patients.

Keywords

Allograft rejection; complement; costimulation; T cells; transplantation

The term “complement” was introduced by Ehrlich in the late 1890s to describe a heat-sensitive component of normal human serum responsible for complementing the antimicrobial activity of antibodies. We know now that the complement system consists of a set of soluble and membrane-bound proteins that constitute approximately 15% of the globulin fraction.¹ Complement activation can mediate opsonization/phagocytosis of pathogens/pathologic particles and direct killing of pathogens/foreign and aberrant cells, while inducing local and systemic inflammatory responses. All of these functions are vital for fighting infection and removing dead cells and foreign agents, making complement a crucial element of innate immunity to ensure host defense.¹

Several unexpected observations since 2002, including the finding that C3-deficient mouse kidneys are accepted by wild-type (WT) allogeneic hosts with normal serum complement activity,² have resulted in paradigm-shifting insights into how the complement cascade can influence tissue inflammation and adaptive immunity.

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Several research groups have shown that complement produced and activated by parenchymal cells modulates ischemia-reperfusion injury³ and can contribute to the development of tissue fibrosis.⁴ Moreover, T cells and antigen-presenting cells (APCs) produce complement components and up-regulate expression of complement receptors during cognate interactions.^{5,6} The local expression and activation of complement provides critical signals that promote T-cell activation and maturation. More recently, evidence also has been generated showing that local complement inhibits regulatory T-cell generation and function.^{7,8} In the present review, we provide perspectives for how these mechanisms are involved in the pathogenesis of transplant rejection, how they apply to human beings and how targeting these pathways potentially could benefit clinical outcomes.⁴

OVERVIEW OF THE COMPLEMENT CASCADE

The complement system comprises more than 30 soluble and membrane-bound proteins that are activated in a cascade-like fashion by three pathways: (1) the lectin pathway, which is triggered by carbohydrates present on the bacteria surface; (2) the classical pathway, which recognizes complement-fixing immune complexes; and (3) the alternative pathway, which is activated continuously at a very low level through spontaneous complement hydrolysis.

The three activation pathways converge at the formation of C3 convertases, multimeric protein complexes with enzymatic activity (Fig. 1A).¹ Cleavage of C3 yields C3a and C3b, the latter of which triggers formation of the C5 convertase. Subsequent C5 cleavage initiates formation of the membrane attack complex (C5b-9) on the target cells, leading to their lysis. 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 such as macrophages and neutrophils.⁹

Complement activation is a tightly regulated cascade designed to prevent bystander damage to host cells.⁹ This regulation is accomplished through secretion and expression of multiple soluble and membrane-bound complement regulatory proteins. Decay accelerating factor (DAF or CD55) is a glycosphosphatidylinositol-anchored, membrane-bound, complement regulatory protein that accelerates the decay of cell-surface assembled C3 convertases. DAF limits downstream complement activation and restricts production of the aforementioned cleavage products.¹⁰ Notably, DAF only functions intrinsically, limiting complement activation on the cell surface upon which it is expressed, but not proximally located pathogens, which lack DAF expression (Fig. 1B). Human CD46 (murine homologue *Crry*), also known as membrane cofactor protein, has similar decay-accelerating function but additionally shows cofactor activity. In conjunction with soluble factor I, this membrane-bound regulator inactivates C3b to iC3b, thereby preventing re-formation of the C3 convertase. Other examples of complement regulators include CD59 (protectin), a cell surface-expressed regulator that inhibits formation of the membrane attack complex at the C9 step and factor H, a soluble complement regulatory protein that shows both decay accelerating and cofactor activity. Through these various checkpoints, complement regulators naturally restrict the deleterious effects of complement activation on self-cells.

The large fraction of the complement components circulating in the blood (systemic compartment) is produced by the liver. However, complement components also can be generated by tissue-resident (eg, tubular cells in the kidney¹¹) and migratory cells, such as T cells and APCs.¹² This peripheral source can finely modulate local levels of complement without affecting systemic complement levels or activation.

LOCAL COMPLEMENT REGULATES ADAPTIVE IMMUNITY

Interactions between complement and adaptive immunity were first hypothesized in the 1970s when Pepys¹³ observed that mice treated with cobra venom to clear complement were unable to mount potent antibody responses. Subsequent mechanistic studies have shown that C3dg, a cleavage product of C3b, binds to the B-cell-expressed complement receptor 2 (CR2, CD21) and, through this interaction, lowers the threshold for B-cell activation.^{9,14} The effect of complement on B-cell immune responses raised the possibility that complement C3dg might be used as an adjuvant to enhance the efficacy of vaccines aimed at inducing protective pathogen-specific antibodies also in human beings. Such studies were performed and in some situations confirmed that C3dg could boost antibody formation, although unanticipated complexities may limit its effectiveness in human beings.^{12,15–20}

Work from several groups, including ours, since the mid-2000s showed an unexpected role for complement as a regulator of T-cell immunity. In particular, during cognate interactions between T cells and APCs (macrophages and dendritic cells [DCs]), both partners up-regulate and secrete alternative pathway complement components C3, factor B, and factor D, as well as the common pathway protein C5. We also observed up-regulated surface expression of the C3a receptor (C3aR) and the C5a receptor (C5aR) on both partners during cognate interactions.^{5,6} These *in vitro* experiments were performed in serum-free medium such that the only source of complement was the cells added to the cultures. Experiments later showed that these changes are in part a consequence of costimulatory molecule signaling via CD28/CD80/CD86 and CD154/CD40.⁶ Costimulation also resulted in a transient, down-regulation of cell surface-expressed DAF. These changes in complement gene and protein expression promoted local complement activation on the interacting immune cells, yielding C3a and C5a, which stimulate the T cell and the APC in both an autocrine and paracrine manner via the C3aR and C5aR.^{5,6} With our collaborators in the Medof group at Case Western, we showed that signaling via these G-protein-coupled receptors in T cells activates phosphoinositide-3 kinase γ , and induces phosphorylation of the central intra-cellular signaling molecule AKT^{6,12} (Fig. 2). AKT phosphorylation up-regulates the anti-apoptotic protein Bcl2 and down-regulates expression of the pro-apoptotic molecule Fas. We showed that together, C3a and C5a enhance T-cell proliferation and diminish T-cell apoptosis (Fig. 2), explaining the complement-mediated expansion of the effector T-cell repertoire after antigenic stimulation.¹² The evidence also indicates that C3aR and C5aR signaling is required for T-cell homeostasis because T cells deficient in both receptors spontaneously undergo accelerated cell death *in vitro* and *in vivo*.⁶

The immune cell-derived and locally produced C3a and C5a also bind to C3aR/C5aR on APCs, including dendritic cells and macrophages^{6,21–23} (Fig. 2). Our collaborative studies showed that C5aR/C3aR ligation activates the APCs via phosphoinositide-3 kinase γ /AKT, and other investigators have shown the downstream inhibition of cyclic adenosine monophosphate/phosphokinase A, which together activate nuclear factor- κ B. The C3aR/C5aR signals induce up-regulation and release of innate cytokines (eg, interleukin [IL]-12, IL-23) and up-regulation of APC costimulatory molecules (eg, CD80, CD86), further amplifying the immune response and modulating the phenotype toward inter-feron- γ -producing Th1 immunity.^{6,21} Importantly, our group and others showed *in vitro* that APCs deficient in C5aR/C3aR, or in C3, produce less IL-12, express lower levels of CD80, and are weaker T-cell stimulators than WT APCs. Conversely, DCs and macrophages obtained from mice genetically deficient in DAF (*Daf1*^{−/−}, in which restraint on local complement activation is lifted) produce more IL-12 and induce stronger T-cell responses than cells from WT animals.^{6,21–23} Independent of the phenotype of the APC, we showed that T cells deficient in C3aR and C5aR signaling (genetically deficient or blocked) respond poorly to WT and *Daf1*^{−/−} APCs, and undergo accelerated cell death.^{6,12}

To test whether local, immune cell–derived complement or systemic, liver-derived complement regulates T-cell immunity in vivo, we used a bone marrow chimera strategy. T cells in chimeric mice with C3^{−/−} bone marrow (BM)-derived cells did not respond to alloantigenic stimuli despite having normal serum complement, whereas C3-deficient chimeras with WT (C3⁺) BM showed normal T-cell alloreactivity.^{12,18} Analogously, BM chimeras produced using C5aR-deficient donors or recipients confirmed that T-cell immunity is dependent on C5aR expression on BM-derived cells.^{12,18}

COMPLEMENT AND DAF REGULATE BOTH PATHOGENIC AND PROTECTIVE T-CELL IMMUNITY

The effects of immune cell–derived complement are relevant to multiple infectious disease models. Mice lacking C3 show enhanced susceptibility to viral infection,²⁴ whereas *Daf1*^{−/−} animals are better protected and produce stronger T-cell responses to lymphocytic choriomeningitis virus infection than WT controls.²⁰ Animals deficient in both C3aR and C5aR are highly susceptible to herpes keratitis and to *toxoplasma gondii* infection, in the latter case, producing little IL-12 and weak T-cell immunity required for protection from this pathogen.⁶

Complement regulates T-cell–dependent autoimmunity as well. Experimental allergic encephalomyelitis is one model of T-cell–mediated autoimmunity that mimics aspects of human multiple sclerosis. In response to immunization with myelin oligodendrocyte glycoprotein, *Daf1*^{−/−} mice develop more severe paralysis than WT animals, which is associated with stronger autoreactive T-cell immunity, enhanced IL-17 production, and diffuse T-cell epitope spreading.^{6,17,19} These effects are C5aR- and C3aR-dependent because mice deficient in either or both of these G-protein–coupled receptors develop weaker T-cell responses and are resistant to experimental allergic encephalomyelitis, regardless of DAF expression.^{6,19} Studies performed by the Quigg laboratory²⁵ have indicated that complement activation drives autoreactive pathogenic T cells in a model of autoimmune focal and segmental glomerulosclerosis in *Daf1*^{−/−} mice, and that serum-derived C5a interacting with immune cell–expressed C5aR is an essential mediator in an IL-17–dependent model of autoimmune arthritis.²⁶

In 2010 our research group reported that immune cell–derived C3 is required for the induction of T-cell–dependent autoimmune diabetes in mice.²⁷ After documenting that multiple low doses of streptozotocin induces T-cell-dependent, autoimmune diabetes, we showed that coincident with the induced increases in blood glucose levels, alternative pathway complement component gene expression was up-regulated within the islets of the diabetic WT animals. When we repeated the experiments with C3-deficient mice we observed complete resistance to disease, as assessed by the absence of histologic insulitis and the absence of T-cell reactivity to islet antigens. Studies of WT chimeras bearing C3-deficient bone marrow cells confirmed that bone marrow cell–derived C3 and not serum C3 is involved in the induction of diabetes in this model.²⁷

COMPLEMENT AND T-CELL IMMUNITY AFTER TRANSPLANTATION

For many years, the view of complement in organ transplantation was largely confined to the effector functions of complement in antibody-mediated rejection. More recently, evidence has been generated that complement-dependent effects on alloreactive T-cell immunity regulate the phenotypic expression of immune-mediated transplant injury in animal models. In addition to the aforementioned observation that wild-type mice do not reject allogeneic C3-deficient kidneys,² we showed that wild-type mice reject *Daf1*^{−/−} heart allografts (enhances local complement activation) with accelerated kinetics compared with

wild-type grafts.¹⁸ The accelerated rejection of *Daf1*^{-/-} heart transplants is associated with augmented anti-donor T-cell reactivity and is notable in animals devoid of B cells, confirming that local complement activation accelerates graft rejection through a T-cell-dependent mechanism. Moreover, the effect is complement dependent because heart grafts deficient in DAF and C3 show prolonged survival and stimulate weak T-cell responses.¹⁸ By using bone marrow chimeras we then determined that immune cell-derived and/or donor graft-derived complement, but not serum complement, regulate expansion of naive and primed effector, alloreactive, CD4⁺ and CD8⁺ T cells after transplantation. In other work, our group, in collaboration with the Medof laboratory, showed that donor or recipient DAF deficiency accelerates skin graft rejection⁵ and overcomes the immune privilege of the eye,²⁸ enhancing pathogenic T-cell alloimmunity induced by normally tolerogenic corneal transplants, and results in rapid rejection.

Complement and graft versus host disease

Separately, we observed that total body irradiation, a conditioning regimen required to permit engraftment of allo-hematopoietic cell transplantation, caused up-regulation and activation of alternative pathway complement components by recipient APCs. The irradiation-induced complement activation is a critical driver of in vivo alloimmunity in murine models of graft-versus-host disease (GvHD). Allo-hematopoietic cell transplantation with *Daf1*^{-/-} host BM and *Daf1*^{-/-} donor lymphocytes exacerbated GvHD outcome and resulted in expansion of splenic and organ-infiltrating pathogenic T cells. Conversely, T cells deficient in C3aR and/or C5aR responded weakly in allogeneic hosts and showed limited ability to induce GvHD (Fig. 3). By using a clinically relevant treatment strategy, we showed that pharmacologic C5aR blockade reduced GvHD morbidity. Our data mechanistically link APC-derived complement to T-cell-mediated GvHD and support complement inhibition as a therapeutic strategy for GvHD in human beings.

Complement and T cell help

Current concepts regarding how CD4⁺ T cells help alloreactive CD8 cells required for allograft rejection (CD4-deficient mice do not reject cardiac allografts²⁹) are that during cognate TCR/APC interactions, CD154 expressed on CD4⁺ T cells transmits activating signals to APCs through ligation with CD40.^{30,31} This in turn up-regulates costimulatory molecule (CD80/86) and major histocompatibility complex expression on the APC, and induces proinflammatory cytokines (eg, IL-12), which together facilitate optimal CD8⁺ T-cell activation, expansion, differentiation, and survival. Based on our earlier-described findings that downstream effects of costimulatory signals are in part complement dependent, we tested the hypothesis that one molecular mechanism through which CD4⁺ T cells provide help in the induction of alloreactive CD8⁺ T cells engaging a transplant is through stimulating local complement production/activation on the APC. We hypothesized that the resultant C3a/C5a then would activate the CD8⁺ T cell through C5aR/C3aR signaling. We indeed found that in vitro, CD4⁺ T cell help required to fully activate CD8⁺ T cells (as measured by CD8 cell proliferation/ cytokine production) required complement production by APCs and CD8 cell expression of C3aR and C5aR.³² In vivo, recipient DAF deficiency (and specifically DAF deficiency on BM-derived cells) bypassed the need for CD4 cell help, facilitated activation of alloreactive CD8 T cells, and induced cardiac allograft rejection in CD4-depleted or CD4^{-/-} recipients³² (Fig. 4). The effects required complement production by immune cells because CD4-depleted mice deficient in both DAF and factor D did not reject allografts. Our work also showed that DAF deficiency bypassed the requirement for CD40 costimulation to induce CD8⁺ T-cell-dependent allograft rejection.³² Together, these findings support the conclusion that immune cell-derived complement is a molecular intermediary underlying CD4 help required to fully activate alloreactive CD8⁺ T cells.

Complement and effector T cells

In other work we showed that local complement production influences effector CD8⁺ T-cell responses to allogeneic vascular endothelial cells (ECs). After stimulation with interferon- γ , tumor necrosis factor- α , and IL-1, murine ECs produce alternative pathway complement components that activate locally yielding C5a.³³ Experiments performed using in vitro culture systems and in vivo heart transplantation models showed that this EC-produced and locally activated complement regulates T-cell expansion and function. Consistent with the aforementioned in vitro studies, the effects of EC-derived complement are in part transmitted through C5aR signaling on T cells because C5aR deficiency or blockade abrogates responsiveness.³³

Studies published by other investigators have shown that C5a/C5aR interactions are pathogenic mediators of T-cell-dependent kidney transplant rejection in rodents. C5aR blockade prolonged kidney transplant survival in rodents, a result associated with an abrogation of intragraft mononuclear cell infiltration and a diminution in T-cell alloreactivity.³⁴ We showed in 2011 that a blocking anti-C5 monoclonal antibody synergizes with CTLA4-Ig to prevent T-cell priming, limit T-cell trafficking to an allograft, and prolong heart transplant survival in mice.³⁵ Together with observations by other investigators that C5aR blockade prolongs rodent kidney allograft survival,³⁴ these findings support the conclusion that complement is a physiologically important regulator of alloreactive T-cell immunity and a potential target for novel, more selective, antirejection therapies.

LOCAL COMPLEMENT MODULATES REGULATORY T-CELL GENERATION AND FUNCTION

Regulatory T cells (Treg) are essential to maintain immune homeostasis and are critical regulators for a variety of immune responses, including tolerance induction and maintenance for organ transplantation.^{36,37} There are two major subsets of Tregs: thymus-derived CD4⁺CD25⁺Foxp3⁺ natural Treg (nTreg) and adaptive or inducible Treg (iTreg) that develop from naive T cells in the periphery under tolerogenic conditions.³⁶ It has been established that Foxp3 is the major transcription factor that determines the fate, identity, and function of Treg.³⁸

Evidence indicates that the level of Foxp3 expressed within a given Treg affects its suppressive capacity. Genetically induced attenuation (50% reduction), but not absence of Foxp3 in nTregs, causes a defect in nTreg suppression, leading to an aggressive autoimmune syndrome in mice,^{39,40} and lower Foxp3 expression in Tregs has been associated with the development of autoimmunity in human beings.^{39,41} Starting from the evidence that Foxp3 expression in nTregs is associated with suppressed AKT phosphorylation,^{42,43} and that signaling of C3a and C5a through G-protein-coupled receptors, C3aR and C5aR, on effector T cells leads to increased AKT phosphorylation,^{6,12} we tested the effects of local complement on nTreg function.

We showed that nTregs express C3aR and C5aR and that enhancing signal transmission via these G-protein-coupled receptors limits nTreg cell function, whereas blocking signal transduction augments in vitro and in vivo suppressive function in multiple model systems. *C3aR*^{-/-}*C5aR*^{-/-} nTregs more effectively prevent autoimmune colitis and skin allograft rejection in vivo than wild-type cells (Fig. 5). Mechanistically, C3aR/C5aR signaling is linked biochemically to phospho-AKT-dependent phosphorylation of the transcription factor Foxo1, which alters nTreg cell function through Foxp3-dependent and -independent mechanisms.^{44,45}

Studies published in 2013 show that genetically absent or pharmacologically blocked C3aR/C5aR signaling on naive murine CD4⁺ T cells augments in vitro and in vivo generation of alloreactive Treg (iTreg) from naive precursors.⁴⁶ Importantly, in vitro-generated, alloreactive iTregs deficient in C3aR/C5aR show enhanced in vivo stability and better protect against fully major histocompatibility complex disparate GvHD in mice.⁴⁶ A late 2012 publication by the Medof group independently showed that absent C3aR/C5aR signaling facilitates iTreg induction/function and provided strong evidence for additional mechanisms, including protein kinase A/cyclic adenosine monophosphate/CREB-dependent inhibition of cellular transforming growth factor- β production by T cells and APCs.⁷ Together, these findings suggest that lack of signaling via anaphylatoxin receptors in CD4⁺ T cells paves the way for commitment to the iTreg lineage, whereas the induction of iTreg cells is inhibited by the addition of C3a and C5a. Altogether, these results underscore the crucial role of complement in modulating adaptive T-cell responses.

COMPLEMENT IN T-CELL ALLOIMMUNITY: DATA FROM HUMAN STUDIES

Most studies on the role of local complement in immune cells have been performed in rodents; data for human beings still are limited. Functionally active C3aR and C5aR have been described on human monocyte-derived DCs,^{47,48} but expression on primary human T cells is controversial^{49–51} and whether C3a/C3aR and C5a/C5aR ligations directly or indirectly influence T-cell function has not been evaluated adequately. Moreover, although current concepts extrapolated from animal data are that activation of serum complement can impact adaptive immunity, whether immune cell-derived complement modulates human T cells remains unclear.

Work published in 2013 newly shows immune cell-derived C3a and C5a ligate their specific, T-cell-expressed receptors and thereby function as costimulatory intermediaries for activation of human alloreactive T cells. In support of this conclusion, resting human T cells express cell surface C3aR and C5aR and up-regulate them during anti-CD3/CD28-induced T-cell activation. Recombinant C3a and/or C5a substitute for anti-CD28 to induce T-cell proliferation, induce intracellular AKT phosphorylation, and bypass the inhibitory effects of costimulatory blockade with CTLA4-Ig.⁵² Our data indicate that the C3a/C5a derive predominantly from DC-produced complement that locally activates during cognate T-cell/APC interactions. We showed that human monocyte-derived DCs synthesize complement proteins, and that knockdown of C3 in DCs decreased C3a/C5a in supernatants of mixed lymphocyte reactions and partially abrogated alloreactive T-cell proliferation. Conversely, DAF knockdown DC augmented local C3a/C5a production and enhanced T-cell proliferation, the latter of which was blocked by pharmacologic antagonists to C3aR/C5aR.⁵²

Our observations that recombinant C3a/C5a costimulates anti-CD3-induced proliferation coupled with an absence of discernible effects of C3a/C5a on DC phenotype or cytokine production during T-cell/APC interactions supports the concept that the DC-derived anaphylatoxins function as paracrine mediators by ligating their receptors on the responding T cells.

Extending the concepts linking C3a/C5a to murine Treg, new data from our group and independently from the Medof group indicate that local complement exerts a role in human Treg induction as well.^{7,46} We showed that pharmacologic antagonists to human C3aR and C5aR augment in vitro generation and stability of human iTregs from naive precursors. By using a xenogeneic model of GvHD induced by adoptive transfer of human peripheral blood lymphocytes into NOD severe combined immunodeficiency (scid) $\gamma c^{-/-}$ recipients, we showed that pharmacologic C5aR blockade enhances human iTreg generation and stability

in vivo, and results in better disease protection.⁴⁶ These clinically relevant, translational findings provide proof of concept that C3a/C3aR and C5a/C5aR ligations are viable targets for facilitating iTreg-mediated tolerance induction in the context of multiple human disease processes, including GvHD, solid organ transplant rejection, and autoimmunity.

In support of the clinical relevance of local complement in alloimmune responses, studies suggest that immune cell-derived and/or graft-derived complement contributes to human transplant rejection. The quantity of RNA message for alternative pathway complement components and complement receptors, including C5aR and C3aR, is higher in human transplant tissue with histologic evidence of rejection compared with noninjured control tissue.^{34,53} Gene expression profiling of human kidney transplants shows higher expression of several complement genes in deceased donor grafts with longer ischemic times, and, interestingly, the complement gene up-regulation correlates inversely with early and late renal function.⁵⁴ In vitro studies performed by other investigators have shown that analogous to the murine findings, human DCs produce complement and C5aR and C3aR signaling seems to be important in DC activation and function.⁵⁵

In another report, donor kidney expression of a specific polymorphic variant of C3 is associated with worse posttransplant outcomes.⁵⁶ However, the precise mechanism through which this mutation alters allograft injury in human transplant recipients remains unclear and an independent study of a disparate and larger patient population could not verify these initial findings.⁵⁷

COMPLEMENT AS A TARGET FOR THERAPEUTIC INTERVENTION IN TRANSPLANTATION

After the encouraging experimental results, the interest of pharmaceutical companies has grown in the development of novel molecules targeting complement proteins and in testing the immune modulating properties of already available compounds.

Treatment with eculizumab (Soliris; Alexion, Inc, Cheshire, CT), a humanized monoclonal antibody directed against human complement component C5, has been approved by the Food and Drug Administration for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, both rare conditions associated with unregulated complement activation. The latter condition, caused by genetic defects in complement regulatory proteins including, among others, factor H, is characterized by the formation of thrombi in the renal microvasculature, leading to renal failure. Because of the high risk of recurrence on the transplanted graft, this condition is a contraindication for solitary kidney transplantation. Eculizumab therapy allows prevention or treatment of disease recurrence in atypical hemolytic uremic syndrome patients with a solitary transplant, also providing proof that this antibody can be used safely in combination with antirejection treatment.⁵⁸

Supported by these data, other investigators have tested the hypothesis that eculizumab could prevent or treat antibody-mediated acute rejection by inhibiting the antibody-mediated formation of C5a and C5b-C9. Consistently, eculizumab treatment was associated with a significantly lower incidence of antibody-mediated rejection in 26 hyperimmune kidney transplant recipients as compared with a historical control group of 51 sensitized patients treated with a similar plasma exchange-based protocol without eculizumab. These results hold promise for other patient populations, including ABO-incompatible kidney (and other solid organ) transplants, and also cross-match-incompatible heart and lung transplant recipients, in whom a high level of anti-donor human leukocyte antigen antibodies often preclude life-saving transplantation.⁵⁹ Moreover, C5 blockade has been used successfully to reverse already established antibody-mediated rejection.⁶⁰ In light of the association

between anti-human leukocyte antigen antibodies and chronic antibody-mediated graft rejection, eculizumab is now being tested in kidney transplantation with donor-specific antibodies to assess if it can retard renal graft function loss (NCT01327573).

Whether eculizumab can control T-cell alloreactivity and could be used as a novel, potentially selective antirejection drug is unknown. Intriguingly, a trial is testing the efficacy of eculizumab in decreasing disease activity in patients with active antineutrophil cytoplasmic autoantibodies vasculitis (NCT01275287). Another ongoing trial is testing whether the orally available complement C5a-receptor antagonist CCX168 can safely control autoimmune response and allow chronic steroid withdrawal in patients with antineutrophil cytoplasmic antibody renal vasculitis and mild to moderate renal involvement (NCT01363388).

Complement antagonists have been advocated also to accelerate renal function recovery after grafting. Indeed, ischemia-reperfusion injury largely is mediated by complement components C5a and C5b-C9.⁶¹ In a mouse kidney transplant model, recovery is strongly dependent on tissue-mediated production of C3, which also can recruit alloreactive T cells and promote graft rejection.⁶² These findings formed the rationale for an ongoing trial testing whether eculizumab accelerates renal function recovery in kidney transplant recipients at risk of delayed graft function (NCT01403389).

An analogue of the human complement-regulatory protein CD35 (also known as CR1), which inhibits all pathways of complement activation by destabilizing the C3 and C5 convertases, has been conjugated to a myristoylated peptidyl tail able to bind and insert into the lipid bilayer of the cell membranes. This agent, mirococept, is able to inhibit complement activity locally and, as suggested by studies in rats, could be used to protect the graft from reperfusion damage in the post-transplant period. A clinical trial has been designed to determine the efficacy of mirococept in preventing ischemia-reperfusion injury in transplanted kidneys.⁶³

Results of these trials may provide evidence of the immunomodulating effects of complement on human T-cell alloimmunity and thus could form the basis for larger studies in transplant patients.

CONCLUSIONS

Although the complement system originally was defined as a serum component that complemented antibodies in the killing of bacteria, it is now appreciated that complement has a multitude of other functions. Emerging data indicate that immune cell-derived complement activation physiologically regulates immune cell survival and proliferation, modulating the strength and phenotype of adaptive T-cell immune responses induced by infection, autoimmunity, and alloimmune transplant rejection. Studies in human beings are confirming the immune-modulating properties of local complement initially identified in mice, and trials are ongoing to understand the potential usefulness of complement inhibitors in organ transplant recipients.

Further studies are needed to exploit how targeting the complement-dependent mechanisms can help in treating and/or preventing disease in human beings, and whether and how complement signaling affects other aspects of cellular immune responses, including memory T cells, one of the major hurdles to the induction of immune tolerance in organ transplantation.

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Figure 1.

Schematic representation of complement activation pathways. (A) Three pathways lead to complement activation: classical, mannose-binding lectin (MBL), and alternative. Binding of immune complexes to C1q,r,s activates the classical pathway whereas binding of mannose-associated serine protease (MASPs) to mannose motifs expressed on bacteria activate the MBL pathway. Subsequent cleavage and assembly of C2 and C4 proteins form the C3 convertase. The spontaneous hydrolysis of C3 on cell surfaces leads to an alternative pathway: C3 convertase dependent on factor B (fB), factor D (fD), and properdin. C3 convertases cleave C3 into C3a and C3b. C3b permits the formation of C5 convertase. C3b has further roles in opsonization and immune complex clearance. C3a and C5a have inflammatory and chemotactic properties. C5b, in conjunction with C6-C9, allows formation of the membrane attack complex and subsequent pathogen lysis. (B) DAF (CD55), is a cell surface-expressed complement regulator that accelerates the decay of all surface-assembled C3 convertases, thereby limiting amplification of the downstream cascade. Reprinted with permission from Kwan et al.⁶⁴

Figure 2.

Schematic representation of complement-mediated effects on T cells and APCs. C3aR/C5aR signaling on both APC and T cells activates the AKT pathway by phosphorylation (among other pathways). AKT activation on the APC stimulates maturation, cytokine production, and B7 costimulatory molecule expression. AKT activation on the T cell directly promotes IFN- γ secretion, reduces susceptibility to apoptosis, and promotes cell proliferation and reduces iTreg generation and stability. In this manner, C3aR/C5aR stimulation directly and indirectly promotes T-cell maturation with an expanded effector repertoire. Modified with permission from Kwan et al.⁶⁴

Figure 3. C3aR and C5aR on T cells regulate GvHD in mice. Weight change of BALB/c recipients of B6 bone marrow plus WT or *C3ar1*^{-/-}*C5ar1*^{-/-} T cells (1.5×10^5 per mouse). * $P < .05$ versus WT. Reprinted with permission from Kwan et al.⁶⁵

Figure 4.

Recipient *Daf1* deficiency results in cardiac allograft rejection despite CD4 depletion. Survival of BALB/c hearts transplanted into B6 WT ($n = 12$), *Daf1*^{-/-} ($n = 5$), CD4-depleted WT ($n = 5$), and CD4-depleted *Daf1*^{-/-} ($n = 6$). * $P < .05$ versus CD4-depleted *Daf1*^{-/-}, ** $P < .05$ versus WT controls. Reprinted with permission from Vieyra et al.³²

Figure 5.

C3aR^{-/-} nTreg cells more efficiently prevent autoimmune colitis and skin allograft rejection in vivo. (A) Weights of animals given CD45.1 Tconv cells alone (closed circles, $n = 3$), T conv cells plus 2:1 CD45.2+ WT nTreg cells (open squares, $n = 10$), or Tconv cells plus 2:1 C3ar1^{-/-}C5ar1^{-/-} nTreg cells (open triangles, $n = 10$). Data are pooled from two individual experiments. (B) BALB/c tail skin graft survival in B6 rag1^{-/-} recipients receiving Tconv cells alone (black, $n = 3$), Tconv cells + WT nTreg (blue, $n = 12$), or T conv cells + C3ar1^{-/-}C5ar1^{-/-} nTreg (red, $n = 12$). * $P < .05$ versus no nTreg cells; ** $P = .05$ versus WT nTreg. Reprinted with permission from Kwan et al.⁸