Invited Review

The role of complement inhibition in kidney transplantation

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Abstract

Introduction and background: The complement system which belongs to the innate immune system acts both as a first line of defence against various pathogens and as a guardian of host homeostasis. The role of complement has been recently highlighted in several aspects of kidney transplantation: ischaemia-reperfusion, antibody-mediated rejection and native kidney disease recurrence.

Sources of data: Experimental data, availability of complement-blocking molecules (mainly the anti-C5 monoclonal antibody, eculizumab) and several trials in human kidney transplant recipients has led to some areas of agreement and some disappointment.

Areas of agreement and controversies: So far, eculizumab has shown great efficacy in treatment and prevention of atypical haemolytic and uraemic syndrome, some efficacy in the prevention of antibody-mediated and so far no efficacy in the prevention of delayed graft function.

Growing points: Among the numerous potentially available drugs potentially interfering with complement, recent focus has been made on C1 blockers in the setting of antibody-mediated rejection with promising results.

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Areas timely for developing research: Complement is now recognized as a major player in transplant immunology, several targets are going to be tested to define precisely which ones may be potentially useful in clinical practice.

Key words: complement, eculizumab, antibody-mediated rejection, atypical hemolytic uremic syndrome, ischemia-reperfusion

Introduction

The complement system belongs to the innate immune system and acts both as a first line of defense against various pathogens and as a guardian of host homeostasis. The role of complement has been recently highlighted in several aspects of kidney transplantation: ischaemia-reperfusion, antibody-mediated rejection and native kidney disease recurrence. Current interest in the complement system has been fuelled by the availability and development of several complement blockers, of which to date, eculizumab, an anti-C5 monoclonal antibody, has been the most extensively used in transplant settings.

The purposes of the present review are threefold: first, to briefly describe the complement system; second, to emphasize the current relative contribution of complement activation pathways to transplanted kidney tissue injuries; and finally, to describe the consequences of complement blockade on several aspects of kidney transplantation.

The complement system

When complement is activated, a cascade reaction is initiated, leading to the cleavage of inert plasmatic components that generate bioactive components (including C3b, C3a, C5a and C5b-9) with various pro-inflammatory, chemo-attractant and cell-damaging functions. Complement activation is tightly regulated to protect host cells from its toxic effects. A set of at least seven proteins present in plasma (C1 INH, C4b-binding protein, factor H and factor I) or present in cell membranes (decay-accelerating factor [DAF], membrane cofactor protein [MCP] and CR1 [CD35]) modulate the complement proteins and protect host cells and tissues from complement damage.1–3

Schematically, the complement system, with three different pathways of activation and multiple bioactive molecules, is a highly complex system that is regulated by processes operating at various levels both in the fluid phase (plasma) and on cell surfaces. The three pathways differ mainly by their recognition targets, namely, antibodies in the classical pathway and carbohydrates in the lectin pathway, and by the permanent low-level activation in the alternative pathway, which is known as the ‘tick over’ phenomenon (Fig. 1).

With regard to the classical pathway, antigen–antibody complexes activate the C1 component, associating a pattern recognition molecule, C1q, and a hetero-tetramer of C1r and C1s proteases.4,5 Hexameric clustering of IgG to the target surface elicits a mechanical stress to the C1 complex that triggers the conversion of C1r and C1s into active proteases and the generation of the classical C3 convertase, i.e. C4b2a, after cleavage of the next two reacting components of the classical pathway, C4 and C2. For example, C1q binds to the Fc portions of the pre-transplant or de novo donor specific anti-HLA antibodies (DSAs), mainly IgGs, particularly IgG1 and IgG3, and activate the classical pathway.6

With regard to the lectin pathway, activation occurs after the binding of five different lectin pathway-specific carbohydrate recognition molecules such as mannose-binding lectin (MBL)- and ficolin-associated serine proteases, particularly MASP2, to carbohydrate residues on microorganisms followed by the subsequent cleavage of complement C4 and C2 and the formation of the classical C3 convertase, i.e. C4b2a. MBL binds to altered self-peptide ligands that arise from pathological conditions such as ischaemia/reperfusion (I/R) injury leading to lectin pathway activation.7
As for the alternative pathway, no specific initiation is needed. It is constantly activated at a low level by the ‘tick-over’ of C3, which collaborates with two proteins, Factor B and Factor D, to lead to the formation of the alternative pathway C3 convertase, i.e. C3bBb. The C3bBb is a short-lived complex, and stabilization of this complex by properdin (the only positive regulator of the complement system) is required to assure efficient host defence.

The convergence point of all three pathways is C3 cleavage into active C3b that is covalently bound to cells and the liberation of anaphylatoxin C3a into circulation. The alternative pathway amplifies the level of C3b deposition of the target and the generation of C3a. This loop is the centre of the complement cascade.

C3b binds to the C3 convertase of the classical or the alternative pathways to form the C5 convertases, which then cleave C5 leading to generation of the anaphylatoxin C5a, the formation of sC5b9 in circulation and the insertion of the membrane attack complex (MAC) on the cell surface.

Of the three complement pathways, the alternative pathway is the most efficient, leading to formation of more than 80% of the MACs on target cells. Anaphylatoxins C3a and C5a recruit phagocytes to the sites of complement activation and generate an inflammatory environment.

Complement amplification depends on the balance between the C3 convertase formation rate, which up-regulates C3 cleavage, and the C3 breakdown cycle, which down-regulates C3 cleavage. To prevent excessive activation, notably of the alternative pathway, the complement system is regulated by fluid-phase and membrane-bound proteins. Factor H (FH), a major regulatory protein in
plasma, efficiently controls the amplification loop of the complement pathways. Factor H binds to C3b and polyanionic surface markers to prevent the formation of the C3 convertase and subsequently induce its dissociation and is a cofactor for C3b proteolysis by Factor I.\textsuperscript{13} C1-inhibitor specifically binds to and inactivates C1r and C1s and therefore tightly regulates classical pathway activation. C4-binding protein, C4bp, increases decay of the classical pathway C3 convertase, and with Factor I, C4bp inactivates C4b to become C4d. The covalently linked C4d at the endothelial surface is an inactive stable degradation product of C4 activation. The cell surface MCP and CR1 proteins limit the number of active convertases by acting as cofactors for factor I-mediated cleavage of C3b and C4b to their inactive products iC3b/C3dg and C4d, respectively. Finally, decay-accelerating factor (DAF) inhibits the assembly or membrane insertion of the MAC complex and, together with CR1, promotes dissociation of the alternative and classical pathway C3 convertases.

**Complement in kidney transplantation**

During the past few years, the role of complement in various aspects of kidney transplantation has been outlined, including the following conditions: ischaemia-reperfusion injury, antibody-mediated rejection, recurrence of native kidney disease such as atypical haemolytic uraemic syndrome, C3 glomerulopathy, anti-phospholipid syndrome and the accommodation phenomenon in ABO-incompatible transplantation.\textsuperscript{2,3} This current interest is mainly due to the availability of drugs that are able to inhibit complement at various steps of its activation.

**Ischaemia-reperfusion injury**

Schematically, ischaemia-reperfusion-induced endothelial injury leads to lectin pathway activation through binding of mannose-binding lectin (MBL) to epitopes exposed on ischaemic tissue. The alternative pathway amplifies this, while the role of the classical pathway remains controversial. Indeed, in kidney biopsies, components of both the lectin and alternative pathways have been identified.\textsuperscript{2,3,14}

Insights gained from mouse models have emphasized the role of complement in determining the extents of the lesions caused by ischaemia-reperfusion injury. The lesions are decreased by complement factor (C3, C5, CFB, C3aR and C5aR) deficiency or depletion but enhanced by complement regulator (DAF, CFH) deficiency. Local synthesis of C3, primarily from the donor tubular epithelium, is essential for complement-mediated ischaemia-reperfusion damage, whereas the effects of circulating systemic C3 are negligible. Animals deficient in C3aR, C5aR or both in renal cells and circulating leukocytes are protected from injury, outlining the roles of these receptors on the pathogenesis of ischaemia-reperfusion injury. Overall, the animal models suggest that ischaemia-reperfusion injury up-regulates the production of complement factors by endothelial, tubular cells and infiltrating cells. Local activation of complement factors C3a and C5a amplifies this local inflammation.

In humans, the involvement of complement is suggested by the following: detection of soluble C5b-9 in deceased-donor, but not living-donor, kidneys; increased expression levels of complement genes in deceased-donor kidneys; brain-death-induced activation of complement; and up-regulated expression of C5aR in renal tubular cells.\textsuperscript{14}

**Antibody-mediated rejection (ABMR)**

Historically, two dates are important for outlining the role of complement in ABMR. First, Patel and Terasaki, in their seminal paper in 1969, demonstrated the deleterious impact of complement-dependent lymphocytotoxic anti-HLA antibodies, which led them to set up the cross-match test, which is currently a reference in transplantation.\textsuperscript{15} Activation of the classical pathway due to immune-complex combining antigens (MHC molecules, ABO-blood group antigens) and IgM/IgG antibodies called donor-specific antibodies (DSAs) start the process of ABMR. Second, in 1991, Feucht et al. described the deleterious impact of the
presence of the complement C4d component that was evident on peritubular capillaries by immunofluorescence. This led to the first definition of ABMR in the Banff classification in which the presence of C4d on peritubular capillaries was mandatory. Freshly activated C4b binds covalently to the activating surface near the site of initial activation. C4 staining is performed by immunofluorescence or immunohistochemistry using an anti-human C4d antibody that recognizes the C4d region of C4 (corresponding to both the active C4b and/or the inactive C4d). Recently, it has been demonstrated with both histological data and gene-transcript analyses that C4d positivity was not absolutely necessary to define ABMR. This led to a new category of ABMR, known as C4d-negative ABMR. Importantly, the intensity of C4d positivity remains useful but is used mainly to grade the severity of ABMR.

C4 is central to activation of the classical pathway, but its role in the transplant outcome is still unclear. Negativity of C4d staining may be due, apart from technical reasons, to a low level of complement classical pathway activation. It cannot be excluded that the genetic diversity of C4 may explain some cases of C4d-negative ABMR due to the hereditarily low level of C4 in circulation. Neither variations in the number of copies of the C4 gene nor the plasma C4 concentration impact the outcome of allograft rejection, which argues against a potential diagnostic benefit from recipient and donor C4 genotyping for risk stratification. Physiologically, endothelial cells are adapted to low levels of activated complement but not to complement hyperactivity. Although the level of DSAs both in historical sera and at Day 0 are predictive of ABMR, recent findings also highlight the influence of complement hyperactivity. The presence of C1q-binding donor-specific anti-HLA antibodies before or during transplantation is an independent predictor of kidney allograft rejection as recently reported by Loupy et al. However, if complement activation triggered by C1q-binding donor-specific HLA antibodies is hampered at the level of C4, it will prevent C3 cleavage and limit complement activation. Hence, the binding of C3b might better reflect the potential of donor-specific HLA antibodies to fully activate the complement cascade than the binding of C1q. In line with this, Sicard et al. recently reported that detection of C3d-binding DSAs may be a better predictor of graft outcome than the presence of C1q-binding DSAs.

Generation of the pro-inflammatory anaphylatoxin C5a is one of the major factors in graft injury, which occurs when C5a attracts inflammatory cells and induces endothelial cells to increase the expression levels of adhesion molecules, such as endothelial-cell selectin (E-selectin), vascular cell-adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1), as well as cytokines, such as interleukin-1 (IL-1) and IL-6, and chemokines. However, the mechanisms linking complement to the histological parameters, observed in allograft rejection and graft loss, have yet to be fully delineated. The top genes in the ABMR molecular score that have been recently identified include VWF endothelial transcripts that are known to be implicated after exposure of endothelial cells to C5b9 at sub-lytic concentrations. In vitro DSA-induced C5b–C9 deposition onto endothelial cells promotes the synthesis and secretion of cytokotns (IL-6), chemokines (e.g. CCL2, CCL5, CCL8 and CX3CL1) and integrins (ICAM1, VCAM1). Moreover, a procoagulant state arises from endothelial cell synthesis of tissue factors, loss of anticoagulant surface heparan sulphate proteoglycans and rearrangements of the cytoskeleton, entailing cell retraction and exposure to the extracellular matrix. Hence, in severe cases of antibody-mediated rejection, thrombotic injury can dominate so that the rejection resembles thrombotic microangiopathy, with diffuse vascular injury and thrombosis.

Recurrence of disease involving mainly the alternate pathway

Atypical haemolytic uraemic syndrome (aHUS) is emerging as the paradigm of a disease caused by inefficient protection of the endothelium from complement attack. Indeed, more than 50% of patients carry mutations in genes for factors H, I, B, C3 and CD46, causing uncontrolled activation of the
alternative complement pathway. In the past decade, a large body of evidence has accumulated to support the dramatic efficacy of eculizumab in controlling the disease and has revealed the possibility of successful renal transplantation in aHUS patients. Before the era of eculizumab, the overall outcome of kidney transplantation in adults with aHUS was poor with a 5-year post-transplant death rate of 7% and graft failure rate of 50% in a large French cohort of aHUS patients. Overall, poor graft survival is largely due to aHUS recurrence, which occurs in 68% of patients. The recurrence risk is major during the first year (with an incidence of 70%) and decreases markedly thereafter. Recurrence is strongly determined by genetic background. Patients with mutations in complement factors have a threefold increase in post-transplant aHUS recurrences compared to aHUS patients without mutations.

C3 glomerulopathy is a heterogeneous group of glomerular diseases associated with acquired or genetic abnormalities in complement alternative pathway components. C3 glomerulopathy is characterized by predominant C3 deposits in the mesangium, along the glomerular basement membrane (GBM) (in cases of C3 glomerulonephritis) or within the GBM (in cases of dense deposits diseases). The clinical presentation is heterogeneous with proteinuria (sometimes with nephritic syndrome), haematuria, hypertension and renal failure. C3 glomerulopathies have a poor renal prognosis with progression to end-stage renal disease in 50% of cases during the first decade after initial presentation. Patients have low serum C3 levels that are attributed to fluid-phase activation of the alternative complement pathway in 50% of cases. Animal models have confirmed the role of excessive C3 activation in the pathogenesis of C3 glomerulopathy. In addition, some patients have increased sC5b9 in the plasma, highlighting terminal pathway activation. To date, the optimal treatment remains unknown. Moreover, the disease recurs after kidney transplantation in ~50% of cases, increasing the likelihood of graft loss.

**Anti-phospholipid syndrome**

Data also suggest activation of complement in anti-phospholipid syndrome, a rare but potentially devastating disease that can recur after kidney transplantation. However, few reports document the role of complement in the development of APS in humans.

**Complement inhibition after kidney transplantation**

It is theoretically possible to inhibit complement activity by blocking (a) the activation pathways, (b) the amplification loop, (c) the anaphylatoxins and (d) the terminal pathway. Currently, there are three available complement inhibitors: a monoclonal antibody directed against C5 (eculizumab) and two plasma-derived C1 inhibitors.

**Compounds blocking the terminal pathway**

Eculizumab is a recombinant fully humanized hybrid IgG2/IgG4 monoclonal antibody that is directed against the human complement component C5. Eculizumab has been engineered to minimize immunogenicity, Fc-mediated functions and complement activation. Eculizumab binds to C5 with a very high efficacy and inhibits C5 cleavage by the C5 convertase. Formation of C5a and C5b-9 is arrested, as are the pro-inflammatory, prothrombotic and lytic functions of complement. Inhibition of complement activation at the level of C5 generates an acquired functional C5 deficiency, and in patients with genetically determined C5 deficiency, the risk of meningitis due to the Neisseria species is increased. Prophylaxis with vaccination and antibiotics is therefore mandatory. However, blockade of the complement cascade at the level of C5 preserves the early components of complement that are essential for opsonization of microorganisms and clearance of immune complexes.

In the kidney transplantation setting, eculizumab has been used mainly to prevent delayed graft function, in acute antibody-mediated rejection and for treatment/prevention of aHUS recurrence and anti-phospholipid syndrome.

With regard to preventing delayed graft function, a randomized, parallel-group, double-blind, placebo-controlled, multicentre study was
performed with two doses of eculizumab administered in an acute setting for the prevention of DGF in adult recipients of deceased-donor kidney transplants who were at increased risk of DGF. A total of 288 patients were treated across North America, South America, Europe and Australia. Unfortunately, a very recent press release disclosed that the primary endpoint consisting of the DGF rate was not significantly different in the two-dose regimen of eculizumab compared to placebo (December 21, 2016, www.alexion.net). Two other studies are currently recruiting patients.

The role of eculizumab in preventing antibody-mediated rejection has been reported by Stegall et al. in a single-centre, open-label study. Twenty-six highly immunized patients were desensitized according to local practice (mainly plasma exchanges) and then transplanted with a living-donor kidney. They received eculizumab on Day −1, Day 0 and then weekly for 1 month and monthly during varying periods of time from 2 months to 1 year. These patients were compared to 51 historical controls that were desensitized but did not receive eculizumab. At 1 year, the incidence of acute AMR was 7.7% in the eculizumab group compared to 41.2% in the historical control group. Graft and patient survival were excellent in both groups. Interestingly, while on eculizumab, screening kidney biopsies were normal, and at 1 year, the incidence of transplant glomerulopathy was 6.7% in the eculizumab group versus 36% in the control group. However, a recent update tempered the enthusiasm about the long-term benefit of C5 blockade in AMR: the incidence of neither transplant glomerulopathy nor subclinical microvascular inflammation was different between the eculizumab-treated and control groups at 1 and 2 years post-transplant. Eculizumab had no impact on the DSA levels. Importantly, the duration of eculizumab therapy greatly varied across patients, from 4 to 39 weeks post-transplant because eculizumab was discontinued whenever the B flow-cross-match channel shift was lower than 200. However, the incidence of subclinical AMR was greater in patients who were still being treated with eculizumab at 3–4 months than in patients who had stopped the drug because of decreasing DSA titres. Together, this set of data suggested that eculizumab efficiently prevented the occurrence of early full-blown AMR yet failed to fully block the progression of subclinical AMR lesions in patients with a persistently high titre of DSA. A randomized trial in living-kidney donor recipients (NCT0199593) was performed in which patients were treated with or without eculizumab over 3 months, along with desensitization according to local practice. This trial, which has not yet been published, did not confirm the conclusions drawn from the previous study. A press release reported that there was no significant difference between the two groups after 9 weeks of follow-up: the primary endpoint rate was 9.8% in the eculizumab group versus 15.7% in the control group. Further analysis and publication of this study are nonetheless warranted because it will be important to determine whether AMR episodes occur right after eculizumab treatment is interrupted. If so, this finding will support a more prolonged treatment. Another study of deceased-donor kidney recipients (NCT01567085) without a control group was also performed in patients with an HLA donor-specific antibody (DSA) with an MFI greater than 3000: the incidence of acute antibody-mediated rejection was 7.5% at 3 months and 10% at 1 year. These AMR rates are quite similar to those of the Stegall study. Other studies are currently ongoing to treat acute clinical (NCT01895127), chronic ABMR (NCT013227573) and ABO-incompatible kidney transplantation (NCT011095887) because to date, only anecdotal reports are available.

Eculizumab has also been used in kidney transplantation to treat or prevent recurrences of aHUS. The efficacy of eculizumab was demonstrated in four prospective, multicentre, single-arm, non-randomized studies involving 100 patients with a follow-up time of at least 2 years. Before the first infusion of the drug, patients had to be vaccinated against meningococcus, and in some countries, such as the UK and France, patients received additional antibioprophylaxis. In both the patients with a very active disease and patients with a more chronic disease who were maintained on long-term
plasma therapy, eculizumab was efficient for increasing platelet counts in ~90% of cases, normalizing haematologic abnormalities, eliminating the need for plasma therapy from the beginning of the study onward and improving quality of life. One of the best results was a significant improvement in estimated renal function with a mean gain of 35.2 ml/min in cases of progressing disease and 7.2 ml/min in patients with long disease durations, with some patients no longer requiring haemodialysis. The drug safety profile was excellent as long as prophylaxis against meningococcal infection was maintained. Interestingly, while haematological abnormalities were corrected in kidney transplant recipients as they were in patients with native kidney disease, the gain in renal function in the recipients was statistically significant but almost similar to that of patients with long disease durations. The reasons for this discrepancy are not entirely clear to date. However, it is reasonable to hypothesize that kidney allografts have poorer recovery from TMA-induced ischaemic damage than native kidneys, as a result of multiple renal ischaemia-induced conditions in post-transplant settings, including CNI toxicity.

In contrast, treatment with eculizumab in patients in ‘real life’ showed better results. The drug was used either to treat patients with overt recurrence or to prevent recurrence during the post-transplant course.

The major findings in kidney transplantation have been reported by Zuber et al.34,35 Twenty-one patients gathered from both an extensive literature search and a French national survey were studied. The patients who received the drug to treat recurrences included two children and 11 adults with a mean age of 32 years (ranging from 6 to 57). Nine patients lost 17 grafts due to recurrences, representing 82% of cases. A complement abnormality was found in 92% of cases, and the median delay between recurrence and treatment initiation was 30 days (from 1 day to 14 months). Haematological normalization was obtained in all patients, while serum creatinine decreased from 295 ± 171 to 135 ± 69 μmol/l during the first 3 months post-treatment. Interestingly, the sooner the drug was given after recurrence, the greater the renal function improvement. In nine patients (six children and three adults, mean age 9 years), eculizumab was used to prevent aHUS recurrences. One patient lost his transplant due to arterial thrombosis, but in all other cases, after a median follow-up time of 16.5 months (4.5–60), patients were recurrence-free, with a mean serum creatinine level of 123 μmol/l (48–238) and excellent renal function (71.6 ± 44.8 μmol/l) after a mean follow-up time of 14.5 months. The main conclusion from this preliminary study of aHUS were that eculizumab was efficient for treating and preventing recurrence after kidney transplantation. A complement workup is not necessary to start treatment but may guide the long-term management and duration of treatment, which is still a matter of discussion.

At our institution, 12 aHUS patients were transplanted using eculizumab prophylactic treatment. This large one-centre study yielded results that were consistent with previous reports (manuscript submitted). No aHUS recurrence occurred as long as the treatment was pursued, but three unexpected features deserve to be mentioned.

In one case, although aHUS was undoubtedly the cause of ESRD, post-transplant screening biopsies at 3 months and 1-year disclosed histological features of C3 glomerulonephritis, including C3 deposits and endocapillary proliferation. This finding indicates that C3G and aHUS share common pathogenic pathways and that C5 blockade does not prevent upstream C3 activation.

In three cases, subclinical and/or chronic AMR lesions were detected in screening biopsies despite long-term eculizumab therapy. This finding was consistent with previous reports showing that C5 blockade did not completely inhibit AMR pathogenesis.

Finally, discontinuation of eculizumab, at ~5 years post-transplant, in a patient with advanced chronic graft lesions and poor renal function led to aHUS relapse 3 weeks later. Prompt eculizumab re-initiation normalized the haematological features but did not allow recovery from terminal graft failure. This finding emphasizes that the risk of relapse can remain high long after transplantation, especially in patients with high-risk mutations.
Eculizumab was also given to several kidney transplant recipients with recurrences of C3 glomerulopathy with inconsistent efficacies and to recipients with anti-phospholipid syndrome, including its catastrophic forms, with promising results.35–39

As an anti-C5 blocker, eculizumab has shown a very convincing efficacy rate in aHUS and interesting results in the prevention of ABMR in kidney transplantation. Thus, it is likely that this compound will remain important in these areas. The issue of cost is, however, a very important one, particularly since kidney transplantation and ABMR are no longer rare diseases but frequent ones.

Compounds blocking the classical activation pathway

DSA-triggered activation of the classical pathway, which goes all the way down to the terminal attack complex, ultimately punches holes into DSA-bound endothelial cells. Although eculizumab efficiently prevents C5b-dependent membrane attack complex formation and C5a release, eculizumab preserves upstream proteolytic cleavage of C3 into C3a and C3b. The very first step of the classical cascade consists of a C1q conformational change leading to activation of the C1r/C1s proteases and subsequent generation of the classical C3 convertase. C3a is a chemotactic agent that may contribute to the recruitment of inflammatory cells into the microvascular bed of the allograft and participate in ABMR pathogenesis. Moreover, experimental models have emphasized the role of complement products in priming and promoting the T cell immune response.40

Supporting the clinical relevance of these observations, T cells from CD55-deficient patients were recently characterized with an enhanced pro-inflammatory profile after T-cell-receptor engagement. Importantly, dual inhibition of the anaphylatoxin receptors C3aR and C5aR reverted the inflammatory burst caused by CD55-deficient human T cell activation back to baseline levels.41 Finally, C1 inhibition reduces the release of chemotactic microvesicles, which are increasingly recognized in the settings of transplantation as a source of persistent donor MHC molecule presentation through the semi-direct pathway, from inflamed tissue.42 Taken together, these data suggest that proximal control of the complement classical pathway should theoretically elicit a more potent effect than eculizumab on both the T-cell and B-cell components of the alloimmune response.

On the other hand, this supposedly greater efficacy might come with a higher toll. Indeed, the lack of specificity of serine protease inhibitors (such as C1INH) implies a broad inhibitory effect on both the classical and lectin pathways that may dramatically reduce C3-dependent opsonization of pathogens and increase susceptibility to infections. However, one must acknowledge that all of these explanations remain unconfirmed to date as the medical literature is still critically lacking any study addressing the superiority of one treatment over the other in ABMR. Another unanswered question regards whether reaching supra-physiological concentrations of C1 inhibitors, through the administration of plasma-derived C1INH, results in parallel improvements in classical pathway regulation in vivo. Importantly, C1INH immunotherapy was originally developed for C1 esterase inhibitor deficiency, and the dose-effect response in supra-physiological concentrations has yet to be demonstrated. In this respect, TNT003, an anti-C1s monoclonal antibody, demonstrated concentration-dependent inhibition of C3b deposition and anaphylatoxin formation.43

Purified C1-esterase inhibitor, licensed in the treatment of hereditary angioedema, was in two studies to either treat or prevent acute ABMR in immunized patients. Tillou et al. first demonstrated the efficacy of blocking C1 with a recombinant C1 inhibitor used to prevent acute ABMR in alloimmunized baboons.44

In the first study, Ashley Vo et al. performed a Phase I/II placebo-controlled trial of C1-inhibitor for preventing antibody-mediated rejection in HLA-sensitized patients.45 Twenty highly sensitized kidney transplant recipients were desensitized first and then randomized to receive either placebo or plasma-derived human C1 inhibitor (Berinert®, 20 IU/kg/dose) twice weekly for a total
of seven doses. No patient developed rejection during the study in the C1-inhibitor group, but one developed rejection in the control group. Tolerance of the drug was acceptable. Interestingly, C1q-fixing antibodies decreased in the treated group.

More recently, Montgomery et al. used another plasma-derived C1 esterase inhibitor to treat acute antibody-mediated rejection following kidney transplantation in a randomized double-blind placebo-controlled pilot study. The drug was used in nine patients as an add-on to the standard-of-care treatment of acute ABMR (20,000 units in divided doses every other day for 2 weeks) and compared to nine patients without the C1 inhibitor. The treatment was well tolerated. With regard to efficacy, no patient developed transplant glomerulopathy in the treated group based on the screening biopsy at 6 months post-transplant versus 42% in the placebo group. Importantly, concurrent plasma exchanges may have reduced the functional C1 inhibitor level. Thus, there is still room for protocol refinement in any attempt to combine plasma exchanges and the C1 inhibitor.

Finally, plasma-derived C1 inhibitor (Berinert®) was used in a pilot study in patients with acute ABMR who were resistant to the standard-of-care treatment. Although the number of treated patients was low, there was a trend towards better renal function at the end of the follow-up period. A randomized phase-3 trial is currently underway to confirm and extend these promising results.

Notably, there is an ongoing trial examining the use if the C1 inhibitor to prevent delayed graft function.

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**Fig. 2** It is possible to inhibit complement activity by interacting with some pivotal complement fragments, such as C3b (Compszatin or cp20), C5 (as Eculuzumab), Factor B or Factor D (humanized Ab again FB or FD), which increases the inhibitory capacity of regulatory proteins with soluble recombinant versions of membrane-bound proteins, such Factor H (mini FH, TT30), CR1 (sCR1, TT10, APT070 or Mirocept) or serine protease inhibitors (C1 inhibitor or anti-MASP2 and anti-OMS872). Therapeutic blockade of anaphylatoxin effects with a C5a receptor (CD88) antagonist is challenging for modulation of inflammation (References in46).
Conclusion

There are currently more than 20 compounds that may interfere with the complement cascade at various levels. Complement offers many intervention points from proteases that drive the activation cascade to anaphylatoxins that mediate the inflammatory responses (Fig. 2). It is theoretically possible to inhibit complement activity by either interacting with some pivotal complement fragments, such as C3b (Compsstatin or cp20), C5 (as Eculizumab), Factor B and Factor D (humanized Ab again FB or FD) or increasing the inhibitory capacity of regulatory proteins with soluble recombinant versions of the membrane-bound protein, such Factor H (mini FH, TT30), CR1 (sCR1, TT10, APT070 or Mirocept) and serine proteases inhibitors (C1 inhibitor or anti-MASP2, OMS872). Therapeutic blockade of the anaphylatoxin effects with the C5a receptor (CD88) antagonist (CCX168) may modulate inflammation. The C5a receptor antagonist was recently developed to inhibit inflammation caused by C5a anti-neutrophil cytoplasmic antibody (ANCA)-associated renal vasculitis (AARV). The complement 5a receptor has been an attractive therapeutic target for many autoimmune and inflammatory disorders. Microvascular inflammation is a key lesion of ABMR, and C5a may be regarded as a major inflammatory peptide. C5a or C5aR blockade successfully attenuates organ damage induced by ischaemia-reperfusion (IR) injury. The C5a receptor is expressed by immune cells (neutrophils, macrophages, and dendritic cells) and vascular endothelial cells. The C5a receptor antagonist may be an alternative or additional therapeutic option to block transplantation-associated inflammatory events. Targeting the response to C5a with a C5aR antagonist would selectively quench the cellular damage without directly affecting the formation of the terminal complement complex, which is known to be required for resistance to encapsulated bacterial infections, such as Neisseria meningitis.

However, not all of them will be tested in transplantation.

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Conflict of interest statement

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References


