The multifaceted role of complement in kidney transplantation

*Ali-Reza Biglarnia[1], Markus Huber-Lang[2], Camilla Mohlin[3], Kristina N. Ekdahl[3,4] and Bo Nilsson[4]**

Abstract | Increasing evidence indicates an integral role for the complement system in the deleterious inflammatory reactions that occur during critical phases of the transplantation process, such as brain or cardiac death of the donor, surgical trauma, organ preservation and ischaemia–reperfusion injury, as well as in humoral and cellular immune responses to the allograft. Ischaemia is the most common cause of complement activation in kidney transplantation and in combination with reperfusion is a major cause of inflammation and graft damage. Complement also has a prominent role in antibody-mediated rejection (ABMR) owing to ABO and HLA incompatibility, which leads to devastating damage to the transplanted kidney. Emerging drugs and treatment modalities that inhibit complement activation at various stages in the complement cascade are being developed to ameliorate the damage caused by complement activation in transplantation. These promising new therapies have various potential applications at different stages in the process of transplantation, including inhibiting the destructive effects of ischaemia and/or reperfusion injury, treating ABMR, inducing accommodation and modulating the adaptive immune response.

[1]*Department of Transplantation, Skåne University Hospital, Malmö, Lund University, Lund, Sweden.*

[2]*Institute for Clinical and Experimental Trauma-Immunology, University Hospital of Ulm, Ulm, Germany.*

[3]*Centre of Biomaterials Chemistry, Linnaeus University, Kalmar, Sweden.*

[4]*Department of Immunology, Genetics and Pathology (IGP), Rudbeck Laboratory C5:3, Uppsala University, Uppsala, Sweden.*

**e-mail: bo.nilsson@igp.uu.se*

https://doi.org/10.1038/s41581-018-0071-x

Since the first successful attempts at organ transplantation in genetically identical twins in the 1950s, this procedure has evolved into a routine clinical practice for a large population of individuals with numerous types of life-threatening end-stage organ failure[1,2]. A key element that enabled progress in transplantation was the development of effective immunosuppressive strategies that target adaptive immunity and in particular the T cell response[3]. In kidney transplantation, the use of such strategies has led to a progressive decline in the incidence of early T cell-mediated rejection and to an improvement in overall transplantation outcomes[4]. Indeed, current therapeutic regimens are so effective that pure T cell-mediated rejection now rarely occurs more than 10 years after kidney transplantation[5,6]. However, despite an apparent reduction in the incidence of T cell-mediated rejection over time, major improvements in the outcomes of kidney transplantation have mainly resulted from improvements in short-term outcomes, whereas substantial improvements in long-term outcomes have been difficult to achieve[7,8].

The process of transplantation consists of sequential events that may affect the graft, including brain or cardiac death in deceased donors, the procedures of organ procurement, preservation and reperfusion, post-transplantation immune responses and non-allogeneic insults such as drug toxicity, diabetes, hypertension and recurrent diseases. During this stepwise process, various immune responses can potentially induce graft injury and contribute to premature loss of kidney function. The importance of the early inflammatory response in different situations is evident from the finding that the outcomes of HLA-unmatched, unrelated, living donor transplantation are similar or superior to those of HLA-matched deceased donor transplantation[7–9]. Furthermore, ischaemic insults to deceased donor grafts, with concomitant early graft dysfunction, are closely associated with a substantially increased risk (up to 41%) of long-term kidney graft failure[10].

Increasing evidence indicates a previously largely unrecognized role of the complement system — a central effector mechanism of the innate immune response (FIG. 1) — in the nonspecific and specific inflammatory reactions that occur before, during and after transplantation[11–16]. In this Review we highlight the role of complement in these adverse events and discuss current and future strategies to regulate complement activation and potentially improve outcomes in kidney transplantation.

Complement activation in transplantation

The process of transplantation involves critical events that occur in transplant candidates, in deceased donors, in allografts and in recipients, and potentially damage the graft (FIG. 2). These events trigger an early

Key points

- Complement activation in the donor, the graft and the recipient before, during and after transplantation is a major cause of damage to the kidney transplant.
- Ischaemia and subsequent reperfusion of the graft is the most important mechanism that triggers complement activation; reperfusion is generally regarded as the most detrimental phase of the transplantation process.
- Following transplantation, complement has a role in innate immunological and inflammatory processes that further damage the graft and result in a gradual decrease in its functional mass.
- Complement-targeted strategies might have a role in optimizing graft quality as well as in the treatment of antibody-mediated rejection, induction of accommodation and modulation of the adaptive immune response.
- Promising data from preclinical and clinical studies suggest that complement-targeted therapies could potentially become part of the standard of care for kidney transplantation.

Endotheliopathy
Disorder of the endothelial layer leading to morphological changes of the glycocalyx, exposure of intercellular adhesion molecules and changes in the global function of the endothelium.

inflammatory reaction that commences before and independently of the donor-specific adaptive immune response in the recipient[11]. Major insults that inevitably occur during these initial processes are primarily caused by ischaemia and subsequent reperfusion, which drive the initial inflammatory process of which complement activation is a major effector mechanism[16]. These initial critical events are closely associated with the occurrence of delayed graft function, which in turn is linked to increased allograft immunogenicity and premature graft loss[17,18].

Activation in transplant candidates

Complement components are expressed in the normal kidney (BOX 1) and complement activation in kidney transplant candidates can occur as a result of complement-driven kidney diseases (including C3 glomerulopathy, membranoproliferative glomerulonephritis type I, atypical haemolytic uraemic syndrome (aHUS) and IgA nephropathy (IgAN)) and/or conditions such as diabetes and dyslipoproteinaemia[19]. In addition, many patients who are waitlisted for kidney transplantation undergo maintenance haemodialysis until a suitable transplant becomes available. During haemodialysis, contact of the blood with the biomaterial surfaces inside the tubing and on the dialysis membranes in the extracorporeal circuit leads to activation of the complement system[20,21].

Recognition of negatively charged surfaces, such as those that are present within the extracorporeal circuit, by complement component C1q and the complement regulator properdin leads to activation of the classical pathway and the alternative pathway of the complement system, respectively[21]. Similarly, ficolin 2 and mannan-binding lectin serine protease 2 (MASP2), which are involved in activation of the lectin pathway of the complement system, are enriched on modern dialysis filters[22]. On the non-charged hydrophobic surfaces of these modern filters and tubing sets, adsorption is the key to recognition by complement components[23]. Material surfaces become covered by an 8 nm film of plasma proteins (approximately corresponding to a monolayer) within seconds of exposure to blood and/or plasma[24]. Proteins that assume an altered conformation and activity upon contact with surfaces include C3, which acquires the ability to activate the alternative pathway[25], and non-antigen-bound IgG, which acquires the ability to activate the classical pathway[26].

In a non-human primate (NHP) model, the levels of complement activation markers increased after dialysis but subsequently returned to normal levels[20], suggesting that complement activation declines after the procedure. However, repeated activation of complement during haemodialysis leads to the generation of inflammatory activation products and leukocyte activation, resulting in systemic inflammation, which is associated with endotheliopathy[21]. This systemic inflammation is likely to support and amplify inflammatory reactions induced by other mechanisms. The use of low-complement activating filters for haemodialysis and the avoidance of ultrafiltration in transplant candidates might reduce the risk of delayed graft function after transplantation[27].

Activation in organ donors

In clinical transplantation, the donor population includes living donors, deceased brain death (DBD) donors and deceased cardiac death (DCD) donors. These categories characterize the condition of the donor and thereby define both graft quality and outcome.

Healthy individuals have a tremendous overcapacity for kidney function that is clearly demonstrated by the observation that kidney donation (that is, a 50% reduction in the total renal mass) does not substantially affect the long-term prognosis or life expectancy of the donor[28–30]. An important reason why long-term kidney function is preserved in living donors is that unlike the graft, the remaining kidney is not subjected to sustained or recurrent immunological and non-immunological injury (for example, drug toxicity) during and after the transplantation procedure.

In living donation, organs are obtained from carefully evaluated healthy individuals and transplanted within a meticulously planned and synchronized surgical environment, resulting in a short ischaemia time. By contrast, deceased donors frequently have pre-existing medical conditions, and deceased donor transplantation is closely associated with substantially longer warm (for DCD organs) and cold ischaemia times (for DCD and DBD organs) than for living donor transplantation.

In contrast to tissues from living donors, those from DCD or DBD donors are exposed to extensive physiological changes, including haemodynamic instability, warm ischaemia, hormone dysregulation and inflammatory responses, leading to an altered cell phenotype in the graft that may result in the activation of complement. Consistent with this concept, levels of complement activation products such as C3d,g and soluble C5b-9 (sC5b-9) are systemically increased in DBD and DCD donors compared to those in healthy individuals and are associated with acute rejection in the recipient[31–33]. In DCD donors, chronic pre-existing comorbidities that lead to death (for example, atherosclerosis causing heart infarction, chronic endotheliopathy and inflammation) are associated with complement activation while the donor is still alive.

Patients who experience polytrauma followed by brain death are also considered for organ donation. Early

Glycocalyx
A glycoprotein and glycolipid shield that protects the membranes of endothelial cells and other cell types.

systemic activation and depletion of coagulation and complement factors has been found after polytrauma, particularly in patients who later died from their injuries[34]. Furthermore, haemorrhagic shock after polytrauma is a major cause of ischaemia and a driver of systemic complement activation, cellular injury, endotheliopathy, breakdown of the glycocalyx, acute kidney injury and multiple organ dysfunction[34–37].

Various studies have shown that the main C3 allotypes of the donor (that is, C3S and C3F) might influence the outcome of transplantation[38,39]. Although contradictory results have been reported, the C3F form seems to be protective[38]. This conclusion was supported by the findings of a subgroup analysis of DCD donors, which identified an independent inverse association of the donor C3F allotype with primary non-function of the graft[39]. Although these preliminary data warrant further validation[38,39], they suggest that C3-targeted complement regulation before, during and after organ collection could reduce tissue damage and improve both the quality of the graft and the outcome of transplantation.

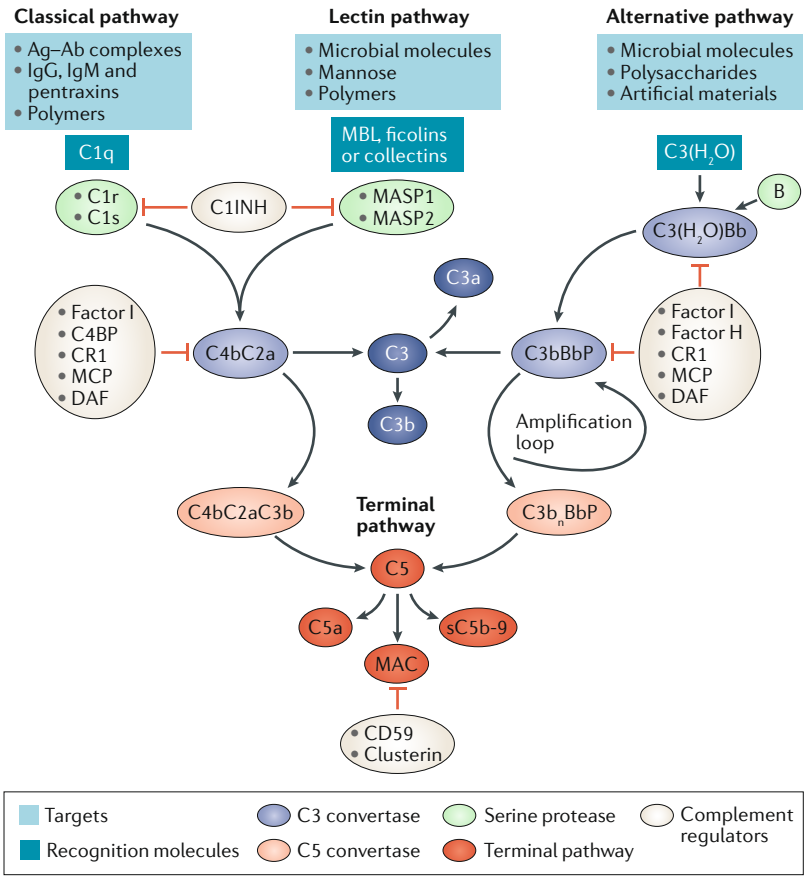

Fig. 1 | **Overview of the complement system.** The complement system can be activated by the classical, lectin and alternative pathways. Recognition molecules within these pathways bind to structures present on pathogens and other target structures, and initiate the assembly of the C3 convertases C4bC2a (classical and lectin pathways) and C3bBbP (alternative pathway), which cleave C3 to generate the opsonin C3b and the anaphylatoxin C3a. The alternative pathway includes a potent amplification loop that increases the generation of C3bBbP. The activation pathways converge into a common terminal pathway of which the first step is the proteolytic activation of C5 to form the anaphylatoxin C5a and the fragment C5b, which forms the nucleus of the C5b-9 complex. C5b-9 can assemble in the cell membrane in the form of the membrane attack complex (MAC) or remain in the fluid phase as soluble C5b-9 (sC5b-9). C3a and C5a mediate the recruitment (via chemotaxis) and activation of polymorphonuclear leukocytes by binding to C3a anaphylatoxin chemotactic receptor (C3aR) and monocytes by binding to C5a anaphylatoxin chemotactic receptor 1 (C5aR1) and C5a anaphylatoxin chemotactic receptor 2 (C5aR2) expressed on these cells (not shown). Complement activation is strictly regulated by fluid-phase and membrane-bound inhibitors at multiple levels. Ab, antibody; Ag, antigen; CR1, complement receptor 1; C1INH, C1 inhibitor; C4BP, C4b-binding protein; DAF, complement-decay accelerating factor (also known as CD55); MASP1, mannan-binding lectin serine protease 1; MBL, mannose-binding lectin; MCP membrane cofactor protein (also known as CD46). Figure adapted with permission from REF.[21], Springer Nature Limited.

Activation induced by ischaemia. Ischaemic events are a common cause of organ damage in the donor as well as during preservation and transportation of the kidney graft. During ischaemia the tissue is deprived of oxygen that is transported to the organ by the circulation, and metabolism switches to an anaerobic state. Ischaemia activates complement by several mechanisms, including by lowering the pH of the blood as a result of anaerobic metabolism. The resulting acidic conditions interfere with the complement system, and its altered regulation facilitates activation of the alternative pathway[40]. Of note, in neutrophils the anaphylatoxin C5a can activate sodium–proton exchangers and enhance glycolytic flux, leading to the generation of an acidic extracellular microenvironment[41]. This mechanism may represent a feedforward loop for local complement activation. Anaerobic metabolism also generates NH_3, which is a nucleophile that can break the thiol ester of C3 and thereby act as an initiator of the alternative pathway by forming $C3(NH_3)Bb$ convertases[42].

Ischaemia also leads to a change in the phenotype of endothelial and parenchymal cells, which is recognized by the innate immune system. The endothelial cell surface is constitutively antithrombotic and has anti-complement properties, mainly because of the proteoglycans that form a protective glycocalyx layer[43]. Ischaemia induces expression of heparanase and metalloproteinases in the endothelial cells of the vessel wall, which results in the cleavage and breakdown of the glycocalyx[44]. This breakdown results in the loss of regulators of the complement, coagulation and contact systems, including antithrombin, activated protein C, tissue factor pathway inhibitor, C4b-binding protein, factor H, and C1 inhibitor (C1INH), which are released from the cell surface. The loss of these regulators means that the endothelial cell surface is no longer protected from attack by the complement, coagulation and contact systems (FIG. 3).

During ischaemia and intravascular inflammation the endothelium is activated by cytokines or by insertion of the membrane attack complex (MAC) and thereby converted to a pro-coagulatory, pro-adhesive and pro-inflammatory state[45]. Although the formation of the MAC rarely causes cell lysis, cell damage and inflammasome activation can occur even at sublytic MAC concentrations[46,47]. In addition, during ischaemia, damage-associated molecular patterns (DAMPs) are exposed by hypoxic damaged tubular, endothelial and perivascular cells and can be recognized by pattern

Anaphylatoxin
A complement activation product that can induce a substantial inflammatory response. C3a, C4a and C5a are anaphylatoxins.

Nucleophile
A molecule that donates an electron pair to form a new covalent bond.

Inflammasome
A intracellular protein complex that upon activation induces the generation of IL-1β and inflammation.

recognition receptors such as C1q, mannose-binding lectin (MBL), collectins, ficolins and C3b, leading to activation of all three pathways of the complement system[48,49].

An important study in a murine C3 knockout model demonstrated that C3 synthesis in tubular cells is a prerequisite for rejection of kidney allografts[50]. A subsequent study from the same group showed an increase in C3 levels in murine kidney grafts that was dependent on the duration of the cold ischaemia time[51]. In the donor kidneys, C3 production was stimulated and reached a peak level after reperfusion and contributed substantially to graft damage. Moreover, a study that analysed kidney biopsy samples from kidney donors showed that C3 gene expression before organ procurement was increased in DBD donors compared to levels in living donors[33,52]. This increased local C3 expression in the DBD donors was associated with inferior short-term graft function after transplantation.

Activation resulting from reperfusion. Delayed graft function is often associated with the combined effect of ischaemia and reperfusion[53]. Reperfusion of the ischaemic organ induces a process that is necessary to enable repair of the tissue, but initially causes devastating injury. The rapid oxygenation causes injury via multiple mechanisms, including increased generation of reactive oxygen species[54]. During reperfusion, the unprotected endothelial surface and intravascular cell debris together with local acidosis and hypoxia owing to the effects of the preceding ischaemia trigger and amplify a destructive activation of complement that elicits inflammation[55] (FIG. 4).

Molecules that have been reported to recognize phenotypically altered cells, including endothelial cells, following ischaemia include natural IgM antibodies, MBL and collectin-11 (CL11)[48,49], all of which activate the complement system. Indeed, studies using animal models have provided evidence that complement has an

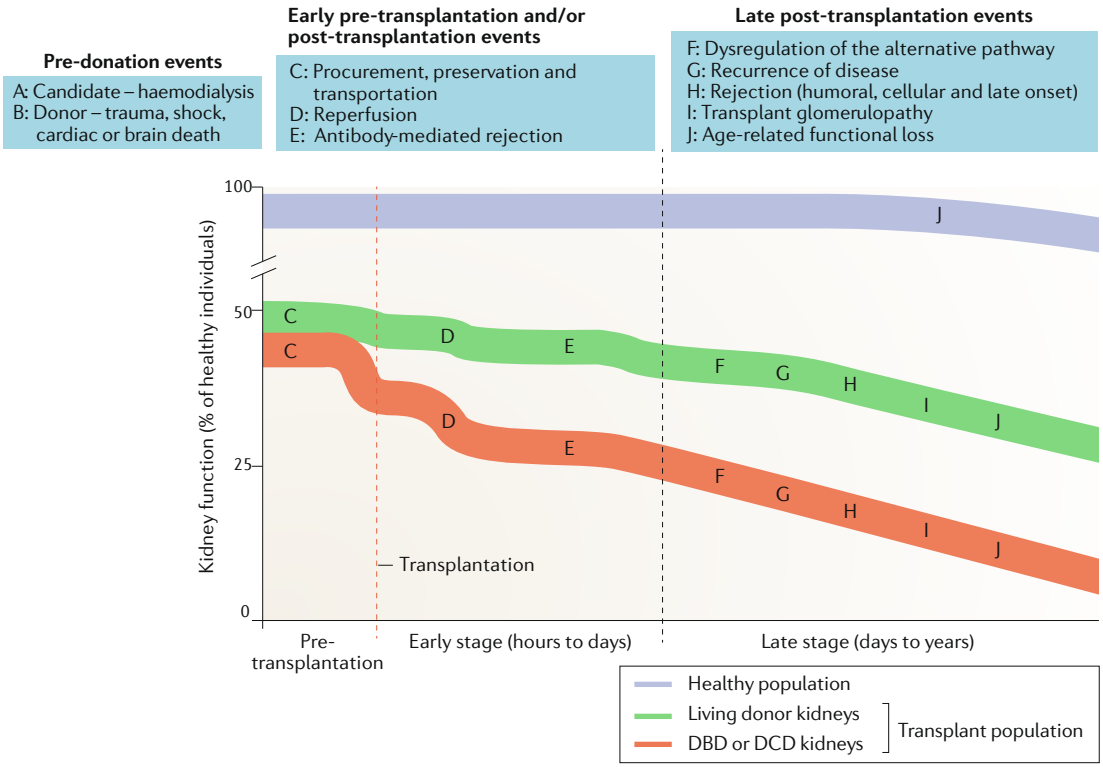

Fig. 2 | **Hypothetical model of complement-related deterioration in kidney graft function.** Sequential events that occur before, during and after transplantation can activate the complement system and lead to loss of kidney function. A: In transplant candidates, complement activation occurs during each haemodialysis session. B: In contrast to living donors, deceased donors experience damaging inflammation and complement activation before and after brain or cardiac death, potentially resulting in reduced viability of the kidney grafts. C: The ischaemia that occurs during transportation and preservation of the graft triggers complement activation. D: Complement activation is further amplified during the reperfusion phase of transplantation. Living-donor transplantation is associated with less ischaemia and consequently less reperfusion injury than deceased-donor transplantation. E: The presence of donor-specific or natural antibodies against the vascular endothelium is a major trigger of complement activation that may initiate rejection. F: In the long term, dysregulation of the alternative pathway of complement in some recipients, for example as a result of mutations in soluble regulators (that may have led to the initial kidney disease), might lead to damage to the glomeruli of the graft. G: Such dysregulation might also contribute to recurrence of the original disease. H: Transplant recipients might also experience rejection, which may be humoral, cellular or late onset. They might also experience I: transplant glomerulopathy or J: age-dependent functional loss similar to that seen in the healthy population. Complement likely has a role in all of these processes with the exception of age-related decline in kidney function. DBD, deceased brain death; DCD, deceased cardiac death.

Alloresponse
An immune response resulting from the recognition of antigens expressed on the surface of cells of non-self origin.

important role in ischaemia–reperfusion (I/R) injury. In porcine renal I/R models, blockade of the lectin pathway or the classical pathway using C1INH before reperfusion protected the kidneys[56,57], whereas in mice blockade of the alternative pathway using a monoclonal antibody (mAb) against C3b before the induction of renal I/R was protective[58]. C4-knockout mice were not protected compared to wild-type controls, suggesting that the classical pathway does not have an important role in renal I/R injury[59]. By contrast, MBL, MASP2 and IgM-knockout mice were protected, suggesting involvement of a lectin-pathway-mediated activation of the alternative pathway, which bypasses C4 (REF.[59]). Consistent with this finding, another mouse study showed that ischaemia in kidney grafts induces exposure of fucose on parenchymal cells and leads to local production of CL11, which recognizes the exposed fucose structures on ischaemic cells[42]. Following reperfusion of the kidney, systemic MASPs become anchored to CL11 and mediate complement activation via the lectin pathway leading to tissue damage. Similarly, an involvement of the lectin pathway and MASP2 has been observed in I/R injury of the heart and brain in mouse models[60,61].

Notably, the relevance of observations in animal models to human transplantation remains to be established. However, consistent with complement activation resulting from ischaemia and reperfusion, kidneys from DBD and DCD donors, but not those from living donors, release substantial amounts of sC5b-9 after reperfusion, indicating that shorter periods of ischaemia are associated with less complement activation[55].

Activation in transplant recipients

Complement activation in transplant recipients occurs as a result of two major mechanisms. Most commonly, complement activation is triggered by IgG and IgM antibodies that are specific for epitopes exposed on donor ABO and HLA antigens. However, the phenotype of the transplant recipient can also lead to complement activation owing to incompatibilities in complement components and/or regulators.

Antibody-mediated humoral rejection. Antibody-mediated rejection (ABMR) is the leading cause of long-term kidney graft loss[5,62]. According to the revised Banff classification from 2013, the histological correlates of ABMR include tissue injury (glomerulitis and/or peritubular capillaritis, acute thrombotic microangiopathy and acute tubular injury), antibody interaction with vascular endothelium (C4d deposition, microvascular inflammation and endothelial-associated transcript expression) and the presence of donor-specific antibodies (DSAs)[63]. The presence or formation of antibodies directed against the vascular endothelium in the graft is a major trigger of complement activation, leading to microvascular inflammation and thrombosis followed by ischaemia, apoptosis or necrosis and finally graft failure.

Complement can contribute to ABMR by several mechanisms (FIG. 5). Activation of C4 results in the generation of C4a, which can bind and activate proteinase-activated receptor 1 (PAR1) and PAR4, leading to the formation of stress fibres in endothelial cells and increased endothelial permeability[64]. Deposition of C3 fragments (C3b, iC3b and C3d,g) on the cell surface promotes the binding of polymorphonuclear leukocytes and monocytes. Although endothelial cells do not express anaphylatoxin receptors (C3a anaphylatoxin chemotactic receptor (C3aR), C5a anaphylatoxin chemotactic receptor 1 (C5aR1) or C5a anaphylatoxin chemotactic receptor 2 (C5aR2))[65], activation of C5 triggers generation of the MAC, which leads to the upregulation and exposure of P-selectin[66] and tissue factor[67] on endothelial cells so promoting thrombotic reactions. Insertion of the MAC also induces cytokine expression[68], apoptosis and necrosis[69,70] and enhances recruitment of allogeneic $CD4^+$ T cells via a mechanism involving activation of the non-canonical nuclear factor-κB (NF-κB) signalling pathway[71].

The humoral alloresponse that is observed in ABO-incompatible and HLA-sensitized transplant recipients is of particular concern because it can result in hyperacute or acute ABMR and a high rate of graft loss[72]. The ABO and HLA systems are the main immunological hurdles in allogeneic transplantation and the binding of anti-HLA or anti-ABO DSAs triggers complement activation, which leads to damage to the transplant.

In vertebrates, antibodies against carbohydrate antigens (for example those within the ABO system) are

Box 1 | **Renal expression of complement components**

Complement proteins and activation products

C3 and its cleavage products iC3b, C3d,g and C3d have been detected in the glomerular and tubular basement membranes and arterioles of the normal kidney[50,179–182]. C4 (C4A and C4B isoforms) is present in the arteriolar walls of the glomeruli and in mesangial cells[183], and C4b-binding protein is expressed in the mesangial cells, glomeruli and the subendothelial layer of the glomerular basement membrane[184]. Transcripts for C3, C4, C2 and factor H have been found in the cortical tubules, whereas factor D and properdin are highly expressed in glomeruli and factor B is expressed in the medulla[185].

Regulatory proteins

Membrane-associated complement regulators that protect autologous cells from complement attack are abundantly expressed in various combinations in different segments of the nephron. Membrane cofactor protein (MCP; also known as CD46) and complement-decay accelerating factor (DAF; also known as CD55) are regulators of C3 convertase activity that are expressed in the juxtaglomerular apparatus, glomerular capillaries, mesangium, podocytes, basolateral surface of epithelial distal tubules, collecting ducts and peritubular capillaries[186–189]. MCP has also been localized to the epithelial basolateral surfaces of proximal tubules, intermediate tubules and the medullary interstitium[189].

CD59 glycoprotein controls the generation of the membrane attack complex (MAC). This regulatory protein is expressed in the juxtaglomerular apparatus, glomerular cells and basement membrane (epithelial and endothelial sides), proximal and distal tubules, collecting ducts, tubular basement membrane and peritubular capillaries[179,180,189].

The soluble regulator of the alternative pathway, factor H, has been detected in the glomerular basement membrane, mesangial matrix and the tubular basement membrane[179,185], where it likely binds to glycosaminoglycans[190,191].

Complement receptors

Expression of complement receptor type 1 (CR1; also known as CD35) is restricted to podocytes[192]. By contrast, the complement anaphylatoxin receptors C5a anaphylatoxin chemotactic receptor 1 (C5aR1)[193,194], C5a anaphylatoxin chemotactic receptor 2 (C5aR2) and C3a anaphylatoxin chemotactic receptor (C3aR)[195] are expressed in proximal tubules and in podocytes. Their expression can be enhanced by inflammatory mediators such as IL-6 (REF.[196]). Constitutive expression of anaphylatoxin receptors has not been found in primary mesangial cells[195], although it has been described in inflammatory settings[197].

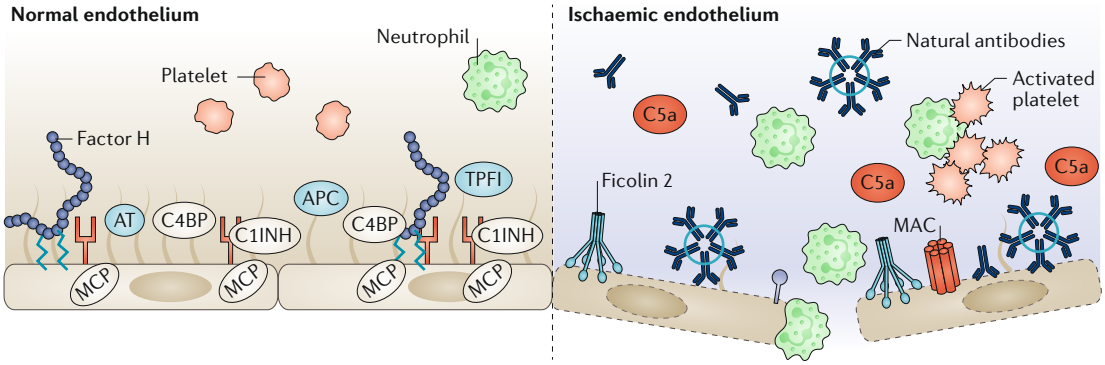

Fig. 3 | **Ischaemia–reperfusion injury in glomerular capillaries.** The normoxic endothelium is protected by the glycocalyx, which has an affinity for and binds the fluid-phase regulators of the complement system C1 inhibitor (C1INH), factor H and C4b-binding protein (C4BP) as well as the coagulation inhibitors antithrombin (AT), tissue factor pathway inhibitor (TFPI) and activated protein C (APC). The endothelium also expresses membrane cofactor protein (MCP; also known as CD46) which inhibits C3 and C5 convertases. The combined effect of these regulators means that the normoxic endothelium is anti-inflammatory and antithrombotic. During ischaemia and reperfusion, the endothelium becomes activated by cytokines and/or complement activation products such as the membrane attack complex (MAC), resulting in the breakdown of the glycocalyx and subsequent loss of regulators of the complement and coagulation systems. In this setting complement activation can be driven by molecules such as ficolin 2 and natural antibodies, resulting in the formation of complexes between activated platelets and polymorphonuclear leukocytes (PMNs) such as neutrophils. Activated PMNs bind to the endothelial cells and migrate through the endothelium into the parenchyma, thereby aggravating the inflammatory response.

produced without previous exposure to the cognate antigen in a T cell-independent manner. These so-called natural antibodies are predominately of the IgM type, but natural antibodies of the IgG3 isotype have also been reported[73]. As IgM antibodies are strong activators of complement, leading to generation of C5a, sC5b-9 and the MAC, the existence of natural antibodies in the recipient against blood group antigens in the graft results in a high risk of acute ABMR but rarely hyperacute rejection[74,75]. Pre-transplantation blood group typing of both donor and recipient is therefore necessary to enable avoidance of ABO-incompatible transplantation. Cryo-electron microscopy studies of the binding of C1q to IgG and IgM have clarified the structural basis of the increased propensity of IgM to activate complement in comparison to IgG[76].

Transplant recipients do not normally have anti-HLA antibodies but immunization, which is T cell-dependent, can occur in response to HLA-mismatched transplants, in multiparous women or in patients who have received blood products such as red blood cells and platelets containing contaminating leukocytes[77]. Transplant recipients with anti-HLA antibodies have an increased risk of hyperacute or acute ABMR and graft loss[78]. Pathological anti-HLA antibodies are often of the IgG class and bind to and activate endothelial cells directly and indirectly, leading to microvascular inflammation and thrombosis followed by ischaemia, apoptosis or necrosis[79]. IgG antibodies can also mediate indirect damage by activating the complement system, which in turn activates leukocytes via the anaphylatoxins C3a and C5a and endothelial cells via the formation of sC5b-9 and the MAC and, unlike IgM, via binding to Fc receptors[79]. The routinely performed complement-dependent cross-matching assay is the gold standard for determining donor and recipient compatibility and has virtually eliminated the risk of hyperacute rejection elicited by preformed HLA-linked cytotoxic antibodies in the clinical setting. However, anti-HLA antibodies and their complement-activating properties are still a major problem in acute and chronic rejection.

Detection of the C4 activation product C4d in kidney biopsy samples has an important role in the diagnosis of humoral rejection[15]. The detecting antibodies are raised against the final fragment of C4 that is generated during physiological cleavage by factor I. The rationale for the use of C4d as a marker of ABMR is that detection of this final cleavage fragment covalently bound to the endothelium indicates activation of the classical pathway of the complement system. However, caution should be taken when interpreting the results of C4d staining in kidney biopsy samples because C4d epitopes are exposed in native C4 and in all covalently bound C4 fragments[80] (FIG. 6). Therefore, the possibility cannot be ruled out that the antibody is detecting C4 that is expressed by endothelial cells in response to cytokine generation rather than C4d that is deposited as a consequence of complement activation[81]. This ambiguity may explain inconsistency in the interpretation of C4d data by different centres. To troubleshoot this redundancy, neoepitope-specific anti-C4d antibodies are currently being developed.

An important advance in the prevention of ABMR was the introduction of Luminex assays using recombinant specific HLA-containing beads, which enable the identification of HLA-specific antibodies that bind C1q and C3d and activate complement. These assays have greater sensitivity and specificity to predict the risk of ABMR than detection of IgG antibodies alone[71–74]. Both the C3d and the C1q-binding capacity of DSAs are associated with a higher risk of kidney graft loss than is the detection of C4d at the time of ABMR diagnosis[82,83].

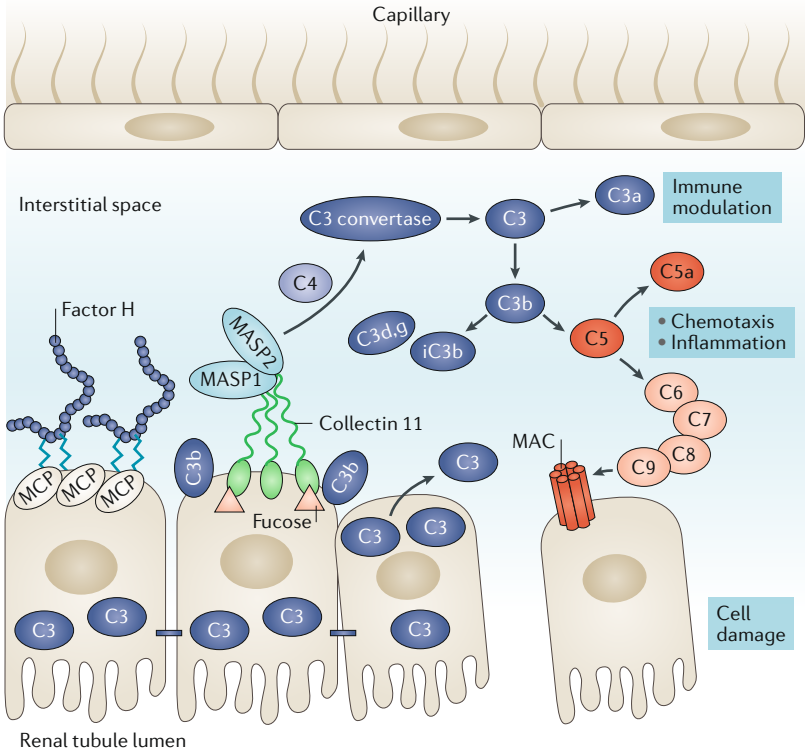

Fig. 4 | **Ischaemia-related activation of the lectin pathway in the proximal tubule.** Ischaemic stress induces the upregulation of fucose and the basolateral secretion of collectin 11 in renal tubule cells[48,49]. In the presence of locally produced complement components or upon reperfusion, mannan-binding lectin serine protease 1 (MASP1) and MASP2 bind to collectin 11 and initiate the lectin pathway of complement activation leading to the release of anaphylatoxins and a destructive inflammatory response that may ultimately result in the formation of the membrane attack complex (MAC) and cell damage. This response is further fuelled by C3, which is locally synthesized in the renal tubular cells in response to ischaemia[51], and by the ischaemia-induced loss of complement regulators such as membrane cofactor protein (MCP; also known as CD46) and factor H, which facilitates complement activation and deposition, cell death and acute kidney injury.

A large prospective study, confirmed that circulating C1q-binding HLA-DSAs, in comparison to non-C1q binding DSAs, are associated with lower estimated glomerular filtration rate (eGFR) and higher levels of proteinuria at the time of antibody detection as well as a higher risk of graft loss at 3 years post-transplantation[84]. In addition, patients with C1q-binding HLA-DSAs had increased microvascular inflammation (as evidenced by accumulation of monocytes, macrophages and natural killer cells in the capillaries of the graft) and increased expression of the inflammatory genes *CXCL11*, *CCL4*, *MS4A7*, *MS4A6A* and *FCGR3A*. These findings suggest the existence of endothelial injury and an inflammatory cell response.

Dysregulation of the alternative pathway. The phenotype of the transplant recipient may predispose him or her to complement activation; in some instances, complement-mediated kidney disease might be the cause of the kidney failure that originally necessitated transplantation. Mutations in genes that encode soluble complement regulators (such as factor H, factor H-related proteins 1–3 and 5, and factor I) and activators (for example, factor B and C3) may lead to dysregulation of the alternative pathway[85]. This dysregulation results in poor protection of the cell surfaces and activation of the alternative pathway in blood plasma, leading to damage to the glomeruli and the development of complement-mediated kidney disease.

Mutations in complement regulators and activators have also been shown to aggravate other inflammatory conditions in the kidney (for example membranoproliferative glomerulonephritis type I–IV and IgAN), although they are not the main driving force behind these diseases[86]. They can also potentially lead to complement-mediated damage in previously unaffected kidney grafts and, in severe cases, recurrence of the original kidney disease (for example aHUS[87], C3 glomerulopathy or IgAN[88]).

Targeting complement in transplantation

As complement activation at various stages of transplantation and thereafter is a serious threat to the graft, complement inhibition is a reasonable approach to improve kidney graft survival and a number of agents are being developed for this application (TABLE 1). In addition, several strategies that use these agents to target different stages of the transplantation process are being investigated. These strategies include optimization of graft quality before transplantation, protection against and/or avoidance of complement-mediated graft damage, induction of accommodation and regulation of the adaptive immune response.

Optimization of graft quality

Preclinical studies have shown that treatment of DBD donors with complement inhibitors is associated with improved graft function after transplantation. This protective effect has been achieved in rats using the specific complement inhibitor soluble complement receptor type 1 (sCR1)[13], which inhibits C3 and C5 convertases, and using purified C1INH[89], which inhibits activation of the classical and lectin pathways of the complement system as well as serine proteases of the coagulation, contact and kallikrein–kinin systems. An ongoing clinical trial is investigating the effect of pre-treatment of DBD donors with C1INH (Cinryze (Shire)) on systemic inflammation and the incidence of delayed kidney graft function in transplant recipients[90].

An alternative and fairly easy clinical approach to complement inhibition in transplantation is modulation of the allograft preservation solution. In a mouse model, C5aR1 blockade in kidney allografts before transplantation significantly improved graft survival[91], suggesting that this intervention might be an effective strategy to reduce complement-mediated damage. However, no clinical data on this strategy are currently available.

Another interesting approach is site-directed complement inhibition targeting areas of C3 fragment deposition or MAC formation at the site of tissue damage and inflammation. Such inhibition has already been accomplished in tissue injury models, for example using the chimeric TT30 (CR2–factor H) molecule[92,93]. In rats, pretreatment of kidneys with TT30 during cold preservation before transplantation inhibited I/R injury[94].

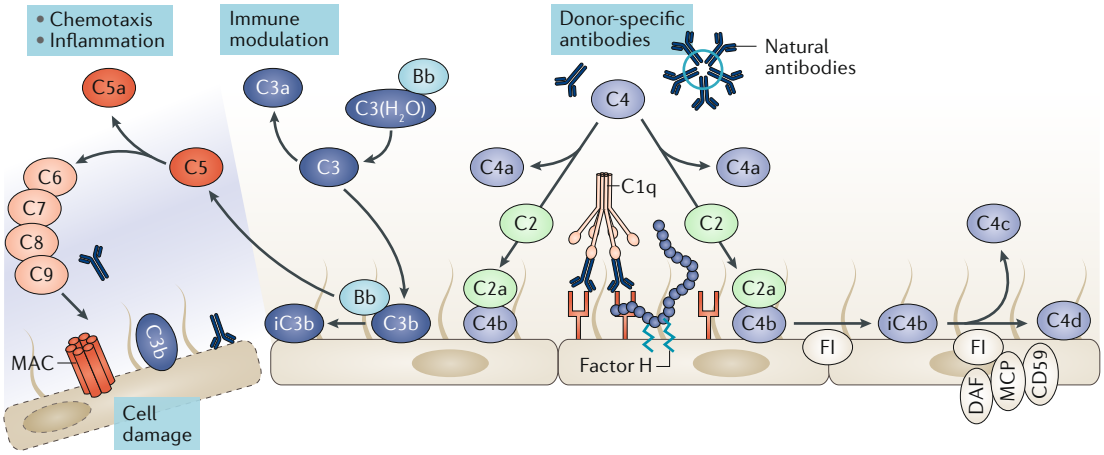

Fig. 5 | **Antibody-mediated rejection in peritubular capillaries.** During antibody-mediated rejection, natural antibodies, for example antibodies against blood group antigens or donor-specific anti-HLA antibodies bind to their targets on the endothelial cells of the graft and trigger activation of the classical pathway of complement via assembly of the C3 convertase C4bC2a on the endothelium. This activation is further amplified by the alternative pathway via formation of the C3 convertase C3bBb, which ultimately leads to formation of the membrane attack complex (MAC). Complement activation is counteracted by the cell-bound regulators complement-decay accelerating factor (DAF; also known as CD55), membrane cofactor protein (MCP; also known as CD46) and CD59 glycoprotein, as well as by factor H, which is present in the blood and on the glomerular basement membrane and inhibits the activation of the alternative pathway. FI, factor I.

Treatment of antibody-mediated rejection

Although a number of candidates for therapeutic complement inhibition are being developed[95,96], only two categories of complement-inhibiting drugs are in the clinic: the humanized anti-C5 mAb eculizumab (Soliris, Alexion) and preparations of C1INH (for example, Berinert (CSL Behring), Cinryze).

Eculizumab specifically inhibits the terminal pathway of complement by blocking cleavage of C5 into C5a and C5b and thus preventing formation of the MAC. This agent is currently approved for the treatment of aHUS, paroxysmal nocturnal haemoglobinuria and refractory myasthenia gravis[97–99] and has also been introduced off-label in the transplant setting for reversal and prevention of antibody-mediated rejection (ABMR) in HLA-sensitized and ABO-sensitized patients[100–109].

The initial experience with eculizumab in sensitized patients with preformed HLA-DSAs undergoing living-donor kidney transplantation showed a reduction in the incidence of ABMR at 3 months in comparison to a historical control group who received post-transplantation plasmapheresis only (7.7% versus 41.2%)[104]. However, at 2 years of follow-up, no difference was observed in the histopathological occurrence of ABMR and in graft survival between the eculizumab-treated patients and the control group[101]. Moreover, in a controlled clinical trial, eculizumab failed to reduce the incidence of ABMR at 9 weeks post-transplantation in comparison to standard of care (SOC)[110,111]. These conflicting findings are further reflected by additional reports, mostly of an anecdotal nature, that show a range of responses to eculizumab from very effective to no effect in patients with ABMR[100–103,105–107,109].

A prospective multicentre study that compared 9 weeks of prophylactic eculizumab treatment to SOC (plasmapheresis and intravenous immunoglobulin (IVIg)) reported that eculizumab was more efficient for early abrogation of ABMR in patients with circulating C1q-binding HLA-DSAs than in those with non-C1q-binding HLA-DSAs[84]. At 3 months, a benefit of eculizumab treatment compared to SOC in terms of lower ABMR incidence was observed only in the patients with C1q-binding HLA-DSAs[84]. Similar findings were previously reported in two patients with non-C1q-binding HLA-DSAs in whom eculizumab therapy was not effective for the treatment of biopsy-verified ABMR with no C4d deposition[100]. These important data suggest that only ABMR that is caused by complement-dependent effector mechanisms might be susceptible to anti-complement treatment. This hypothesis could partly explain the inconsistent results with eculizumab in patients with ABMR as the prevalence of C1q-binding HLA-DSAs was not known in most of the studies published to date[100–109].

Another possible explanation for these inconsistent results is that eculizumab targets the late-reacting terminal pathway of the complement system and does not inhibit upstream complement components. This explanation was supported by the finding of renal C4d deposition in 63% and 32% of eculizumab-treated kidney transplant recipients ($n = 30$) with persistent HLA-DSAs at 1 month and 2 years, respectively[101]. These data indicate that (not unexpectedly) complement inhibition at the level of C5 does not prevent upstream complement activity. In line with this concept, cases of IgM-DSA-driven ABMR despite eculizumab treatment were assumed to be triggered by the generation of immune modulators such as C4a or C3a[64,109]. Moreover, in vitro studies have shown that residual terminal pathway activity via the alternative pathway can occur despite eculizumab treatment[112]. In these studies, the level

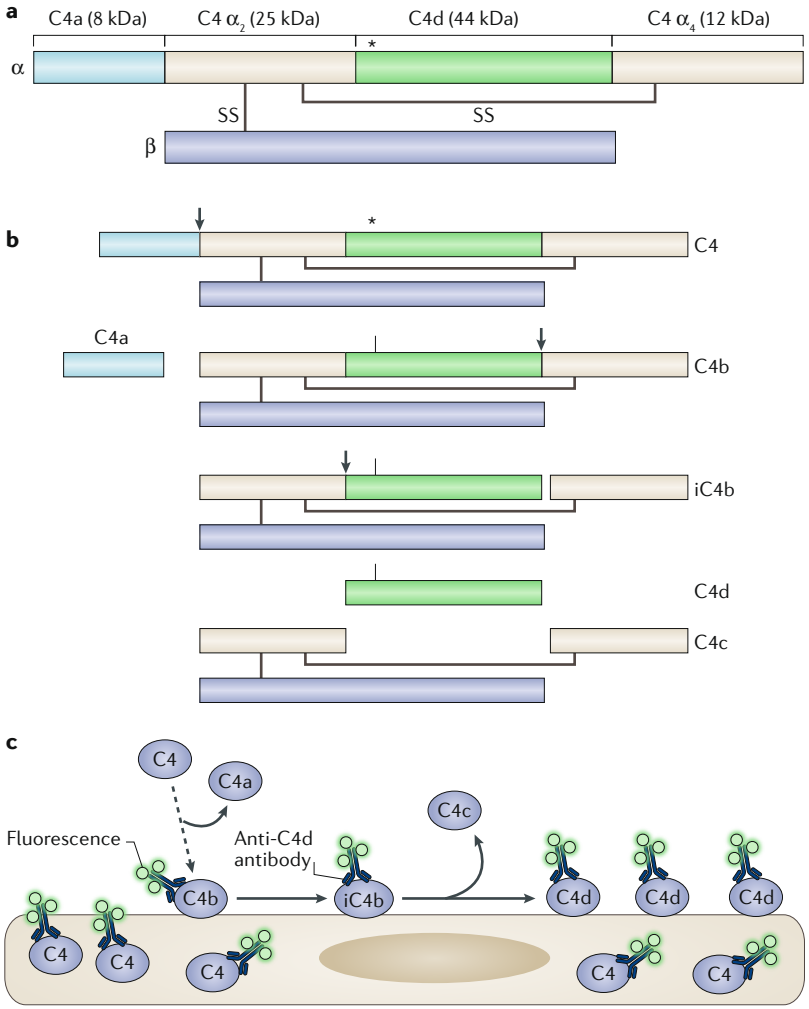

Fig. 6 | **C4d formation and the reactivity of polyclonal anti-C4d antibodies. a** | The structure of human C4 showing the α and β-chains, the positions of the interchain disulfide bonds (SS) and thiol ester (*) and the molecular weights of the fragments produced by proteolytic cleavage. The γ-chain, which is not subject to proteolytic cleavage, has been omitted for clarity. **b** | C4 is digested sequentially first to C4b by the classical pathway convertase C4b2a and then to iC4b, C4c and C4d by factor I[80]. The sites of digestion are indicated by arrows. Activation of C4 leads to disruption of the thiol ester, which subsequently establishes covalent bonds with OH and NH_2 groups on the target surface. **c** | In addition to binding to surface-bound C4d, antibodies raised against this fragment may recognize the same epitope in surface-bound C4b, iC4b and intact intracellular C4. These anti-C4d antibodies will detect all forms of C4 regardless of its activation status.

of residual terminal pathway activity correlated with the degree of complement activation. High C3 turnover as a consequence of strong complement activation can generate densely packed C3b clusters on the cell surface, which in turn can maintain residual C5 activation even in the setting of surplus concentrations of eculizumab[112].

Based on these observations, the concept of upstream complement inhibition is gaining increasing interest. C1INH, either in its recombinant form or as an enriched preparation from human plasma, has been successfully used to prevent allogeneic and xenogeneic humoral immune responses in preclinical models[113,114]. In controlled clinical trials, enriched plasma-derived C1INH has been used for HLA desensitization[115] and for the treatment of ABMR[116,117]. These initial safety and efficacy studies showed that C1INH as an add-on to SOC was a safe and potentially beneficial therapy. In a pilot trial, C1INH was given as a supplement to high-dose IVIg in six patients with nonresponsive ABMR[116]. At 6 months, these patients showed improvements in eGFR compared with levels at enrolment and had less C1q-binding DSAs than did a historical control group. In a randomized, placebo-controlled trial that compared add-on treatment with C1INH to placebo in 18 patients with ABMR who were receiving SOC (plasmapheresis, IVIg and rituximab), no between-group difference in ABMR histopathology or kidney function was observed at 20 days of follow-up[117]. However, a subgroup analysis of 14 patients with 6-month protocol biopsy samples identified no transplant glomerulopathy in the C1INH group, whereas transplant glomerulopathy was present in 43% of patients in the placebo group.

Overall, these initial data could indicate an important role for C1INH as an add-on therapy to SOC for the treatment of ABMR and the prevention of transplant glomerulopathy, which is still the most important risk factor for long-term transplant failure[118]. However, given the exploratory nature of these studies, with a limited number of patients and short-term follow-up, it is too early to draw any solid conclusions. Therefore, larger controlled trials with long-term follow-up are warranted.

An alternative strategy for upstream complement inhibition targets C1s using the humanized anti-C1s mAb BIVV009. A small phase I trial evaluated BIVV009 as a short-course treatment for late acute or chronic ABMR[119]. In 1-month protocol biopsy samples, a marked decrease in C4d deposition was observed compared with index biopsy samples. However, at 50 days post-treatment, no histopathological resolution of ABMR and no change in median eGFR were observed. Longer term follow-up data are not available. BIVV009 has just entered phase III clinical trials for the indication of cold agglutinin disease[120].

A clinically applicable C3 inhibitor, the latest generation of compstatin analogues (Cp40) has been evaluated in a phase I trial in healthy volunteers[121]. However, the results are not yet available. The compstatin family consists of cyclic synthetic peptides that bind to C3 and prevent its activation[122]. Cp40 has a nanomolar IC_{50} and binds C3 with sub-nanomolar affinity[123]. Potential clinical applications of Cp40 include ABO-incompatible kidney transplantation and periodontal disease[96,122].

A totally different approach to attenuate complement activation and binding to Fc receptors uses IdeS, which is a recombinant *Streptococcus pyogenes*-derived endopeptidase. IdeS cleaves all IgG subclasses in the hinge region, first generating an IgG molecule in which one heavy chain is cleaved (that is, a single-cleaved IgG). Cleavage of the second heavy chain generates one $F(ab)_2$ and one Fc fragment. After the first cleavage, the ability of the IgG molecule to bind C1q is lost but its Fc receptor binding ability remains[124,125]. Promising results from a combined phase I–II clinical trial indicated that IdeS reduced or eliminated DSAs and permitted

Endopeptidase
A proteolytic enzyme that cleaves peptide non-terminal bonds within a protein substrate.

Table 1 | **Complement-targeted agents for the prevention of rejection in transplantation**

Compound	Entity	Target	Mechanism	Status	Refs
Intravenous immunoglobulin	Plasma protein	Multiple	Inhibits activation of complement, blocks FcR and C1q	Included in SOC	84,115–117
Rituximab	Humanized mAb	CD20 expressed on B cells	Blocks complement-induced enhancement of antibody generation	Included in SOC	115,117
C1INH	Purified or recombinant protein	C1r, C1s, MASP1, MASP2 and factor B	Inactivates complement serine proteases FXIIa, FXIa and kallikrein; blocks the classical and lectin pathways as a serpin but may also inhibit the alternative pathway by another mechanism	Clinical trials (NCT02502903, NCT01134510, NCT01147302)	90,115,117
Eculizumab	Humanized mAb	C5a	Inhibits C5 cleavage to form C5a and C5b; blocks the terminal pathway	Clinical trials (NCT01567085, NCT00670774, NCT01399593)	85,104,110,111
BIVV009	Humanized mAb	C1s	Inactivates C1s; blocks the classical pathway	Clinical trial (NCT02502903)	119,120
IdeS	Protease	IgG	Digests IgG resulting in loss of C1q binding; FcR binding is retained	Clinical trials (NCT02224820, NCT02426684, NCT02475551)	126–129
APT070 (Mirococept)	Recombinant protein (membrane-targeting truncated CR1)	C3 and C5 convertases	Inhibits C3 and C5 convertases; blocks activation downstream of C3	Clinical trial (ISRCTN49958194)	175–177
Compstatin family inhibitors	Peptide	C3	Binds to C3 and inhibits its cleavage by C3 convertases; blocks downstream activation	Clinical trial (NCT03316521)	121–123
sCR1	Recombinant protein	C3 and C5 convertases	Inhibits C3 and C5 convertases; blocks downstream activation	Preclinical development	13
TT30	Recombinant protein (chimeric CR2–factor H)	Alternative pathway C3 and C5 convertases	Binds to C3d on target cells and inhibits C3 convertases	Preclinical development	92,94
C5aR1 antagonist	Peptide	C5aR1	Blocks C5aR1 so inhibits signalling	Preclinical development	91
Cobra venom factor	Recombinant protein	C3 and C5	Forms stable alternative pathway convertase with factor B that cleaves and depletes C3 and C5	Preclinical development	148

CR2, complement receptor type 2; C1INH, C1 inhibitor; C5aR1, C5a anaphylatoxin chemotactic receptor 1; FXIa, factor XIa; FXIIa, factor XIIa; mAb, monoclonal antibody; MASP1, mannan-binding lectin serine protease 1; sCR1, soluble complement receptor type 1; SOC, standard of care.

HLA-incompatible transplantation in 24 of 25 highly sensitized patients[126–129]. Despite a massive generation of free $F(ab)_2$ and Fc fragments, no serious adverse effects of treatment with IdeS have been observed.

Induction of accommodation

Accommodation is defined as a condition in which the transplant does not elicit complement-mediated rejection despite the presence of DSA and fully functional complement components in the plasma[130,131]. Typically, accommodation is induced in T cell-independent antibody responses, which are elicited by carbohydrate antigens such as ABO and Gal, but accommodation has also been reported for anti-HLA antibodies in vitro and in vivo[132,133] and is suspected to occur in up to 30% of conventional (ABO-compatible) organ transplants[134].

To establish accommodation, low anti-graft antibody levels and/or lowered complement function seem to be required at the time of graft implantation[107,131]. However, the mechanism of accommodation is not known. Overexpression of the genes that encode haem oxygenase 1, A20, BCL-2 and BCL-X has been reported to promote an anti-inflammatory state in NHP models and in transplant recipients[131,135]. The products of these genes regulate the transcription factor NF-κB which in turn downregulates the expression of pro-inflammatory cytokines and chemokines[136].

Many of the processes that are involved in complement-mediated rejection, including apoptosis triggered by tumour necrosis factor (TNF) and necrotic cell death caused by the MAC, can be prevented by pretreatment of endothelial cells with the T helper 2 (T_H2) cytokines IL-4 or IL-13 (REF.[137]). The pathological mechanisms that are associated with this protection involve activation of phospholipid synthesis in association with preservation of mitochondrial structure and function[138] and upregulation of complement regulators such as complement decay-accelerating factor (DAF; also known as CD55) and CD59 glycoprotein in the graft in animal models and transplant recipients[15,139,140]. Other mechanisms that might have a role in accommodation include antigen shedding and repopulation of the endothelial cell lining of the graft with cells from the recipient[141]. However, direct involvement of complement in accommodation is corroborated by the fact that this process can be studied in vitro in complement-dependent systems

in which repopulation cannot occur[137]. Moreover, grafts are often C4d positive, suggesting that in many cases accommodation is induced by factors downstream in the complement cascade.

In the clinical setting, accommodation over the ABO barrier can be induced by various desensitization strategies that aim to establish short-term protection from ABMR by means of B cell depletion (via splenectomy or the anti-CD20 mAb, rituximab) and ABO antibody clearance or inhibition (via apheresis and IVIg in addition to immunosuppression)[142,143]. In a cardiac transplantation model, terminal pathway inhibition using eculizumab induced accommodation in MHC-mismatched and pre-sensitized mice[144,145]. Successful induction of clinical accommodation using terminal pathway inhibition with eculizumab has also been reported[100,101]. The first case involved a donor with blood group type B who was allocated to a kidney–pancreas transplant recipient with blood type A1 and a low isoagglutinin titre at baseline[107]. Within the first week after transplantation the recipient experienced severe ABO-antibody-driven ABMR, which was successfully reversed using short-term eculizumab treatment. The recipient's long-term graft function was normal. Eculizumab has also been used as an add-on treatment in four patients undergoing ABO-incompatible living donor kidney transplantation[108]. In this small series, preserved graft function was achieved in the absence of ABMR at the 12-month protocol biopsy despite prolonged ABO antibody exposure, indicating that accommodation could be induced by terminal pathway inhibition in ABO-incompatible kidney transplantation.

Accommodation has also been induced experimentally using Yunnan cobra venom factor (CVF), which can form an extremely stable convertase, CVF–Bb, which cleaves both C3 and C5 (REF.[146]). As this convertase is not inactivated by complement regulators, treatment with CVF leads to sustained activation of the alternative pathway and consumption or depletion of C3 and C5, which results in temporary inhibition of the complement system. In rhesus monkeys that were presensitized using skin grafts before kidney transplantation, a combination of 2 weeks of CVF treatment and SOC immunosuppression enabled long-term graft survival[132]. In this study, three of five animals treated with CVF maintained normal creatinine levels up to 1,000 days, whereas control animals without CVF lost their grafts after 3 days. Moreover, ABMR was completely absent in the CVF-treated group despite the persistence of donor-specific anti-HLA antibodies. A humanized form of CVF has been developed and could potentially be used as a therapy for ABMR[146]. However, it seems more likely that a C5 inhibitor will be the first compound to be used on a routine basis to induce accommodation in the clinic.

Regulation of adaptive immunity

The complement system has long been considered to act as a bridge between innate and adaptive immunity in the context of transplantation[147]. This concept suggests that the adaptive immune system could be regulated by targeting complement. In the 1970s, studies using CVF showed that complement is involved in humoral immune responses. In these studies, treatment with CVF reduced antibody production in response to immunization and prevented antibody class switching from IgM to IgG after a booster dose[148]. This effect only occurred at fairly low antigen concentrations and could be ameliorated by increasing the antigen dose. In later studies similar effects were obtained in animals that were deficient in components of the classical pathway upstream of C3 (REF.[149]).

In the 1990s, attenuation of the humoral immune response was achieved by blocking complement receptor type 2 (CR2; also known as CD21)[150], which binds to the C3 fragments iC3b and C3d,g when bound to antigens. This receptor is expressed on B cells and follicular dendritic cells and mediates signalling via the adaptor protein CD19, which recruits cytoplasmic signalling proteins to the membrane and thereby decreases the threshold for B cell receptor signalling[143]. Antigens conjugated with repetitive sequences of the activation fragment C3d have been shown to be 1,000-fold more efficient at inducing an antibody response than antigens without C3d,g present (that is, those that only bind to the antigen receptor on B cells)[151]. These studies demonstrate that CR2, in combination with crosslinking of the antigen receptor, is required to induce a humoral immune response and provoke a class switch after a booster dose[152]. These findings suggest that blockade of CR2 is a potential strategy to regulate the adaptive immune response in transplantation.

Increasing data suggest that soluble complement activation products also have a crucial role in transplant-related adaptive immunity, not only by sensing and clearing non-self-danger molecules but also by serving as an important instructor of the adaptive T cell and B cell immune responses[153,154]. Some evidence suggests that intracellularly expressed C3aR and C5aR1 in human $CD4^+$ T cells have a role in several immune functions such as activation of the mechanistic target of rapamycin (mTOR) pathway and NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome[155]. However, a similar expression pattern could not be detected in circulating T cells and B cells in anaphylatoxin receptor reporter mice, suggesting that species differences exist[65,156,157]. Expression of both C3aR and C5aR1 has been reported in murine regulatory T (T_{reg}) cells. In these cells C3aR and C5aR1 drive T_H1 cell maturation, which is associated with the predominant generation of interferon-γ (IFNγ), TNF and IL-2 (REFS[158,159]).

Complement components can also influence T cell responses via the interactions of dendritic cells with $CD4^+$ T cells. Exposure of pathogen-associated molecular patterns (PAMPs) or DAMPs frequently occurs in response to cell stress and inflammation before, during and after transplantation. PAMP or DAMP-induced activation of Toll-like receptors on dendritic cells results in upregulation of C3aR, C5aR1 and MHC class II and the secretion of key complement components such as C3, C5 and factors B and D, which can locally generate C3a and C5a[160]. In turn, these anaphylatoxins can stimulate $CD4^+$ T cells to generate IFNγ and IL-2 and induce effector responses in T_H1 and T_H17 cells[160–162]. IFNγ production

by human $CD4^+$ T cells is also promoted by intracellular C5 activation and stimulation of C5aR1, which is required for assembly of the NLRP3 inflammasome[155].

Remarkably, therapeutic blockade of either C3aR or C5aR1 signalling results in multifaceted alterations in human tolerance to alloantigens. This blockade enhances the generation of induced T_{reg} (iT_{reg}) cells from peripheral $CD4^+$ T cells, supports forkhead box protein P3 (FOXP3) expression, and inhibits iT_{reg} cell conversion to pathogenic TNF and IFNγ-generating effector T cells, all of which lead to iT_{reg}-mediated tolerance and a decreased graft-versus-host reaction[163]. Consistent with these findings, inhibition of C3aR and C5aR1 signal transduction in thymus-derived (natural) $CD4^+$ $FOXP3^+$ T_{reg} cells also results in protective T cell responses and prolonged allogenic skin graft survival, likely through control of FOXP3 expression[158]. Overall, utilizing the anti-inflammatory and immune suppressive functions of FOXP3 T_{reg} cells represents a promising approach to improve kidney graft survival and promote operational tolerance[164]. Ongoing studies are investigating the clinical impact of cell therapy with isolated, ex vivo expanded and re-transfused autologous T_{reg} cells either early after kidney transplantation[165] or in transplant recipients who show signs of inflammation in surveillance renal biopsy samples at 6 months[166]. However, these trials do not include modulation of complement as an intervention or read-out strategy so further investigation is required to determine the role of complement in T_{reg} cell therapy.

Notably, plasma C3 is generated to a substantial extent by the kidneys and seems to be important for the development of memory B cells[167]. Therefore, C3 might have a thus far-unappreciated role in alloantigen tolerance. In regard to the interactions of C5a and adaptive immune responses, C5aR1 blockade initiated before experimental transplantation has been found to significantly reduce the priming of alloreactive T cells in allograft recipients[168]. In a murine transplantation model, C5aR1 deficiency impaired the function of donor and recipient antigen-presenting cells such as dendritic cells and inhibited the response of recipient T cells to allostimulation, which was associated with reduced local inflammation and general renal allograft protection[169].

In summary, systemically and locally produced C3a and C5a result in enhanced antigen presentation and priming of the T cell response, which lead to transplant rejection[170]. Although sC5b-9 formation seems to have an important role in the initial renal ischaemia and reperfusion phase[170], its role in modulating adaptive immunity after renal transplantation is unclear and represents an interesting field for future research. Overall, complement activation is tightly linked to the adaptive immune response after kidney transplantation and complement components are very promising therapeutic targets to modulate adaptive immunity and prevent rejection.

Xenotransplantation

A future strategy to optimize donor organs and ameliorate the shortage of organ donors might arise from xenotransplantation. Genetic engineering of pig kidneys for transplantation to humans or non-human primates avoids recognition of the antigen structures of the graft by natural antibodies of the recipient that would otherwise bind and activate the complement and coagulation systems and induce inflammation[171].

Pigs express complement regulators (CRegs) that are similar to those of humans but do not sufficiently protect pig organs against human complement attack[172]. The introduction of transgenes for the human CRegs membrane cofactor protein (MCP; also known as CD46), DAF or CD59 into donor pigs improved kidney graft survival in NHP recipients from days to weeks[173]. Of note, longer lasting effects of CReg expression on adaptive immunity in transplantation have been reported in the clinical setting — high expression of MCP in kidney biopsy samples during acute T cell-mediated rejection (ATMR) was associated with a 5-year graft survival of 100% compared with only 79% in kidneys with low MCP expression, indicating that a decrease in CReg expression in human grafts is associated with progression of ATMR[174].

Another strategy to avoid complement activation in allogenic and xenogeneic kidney transplantation is to coat the cell surfaces of the kidney ex vivo using a lipophilic linker that enables conjugation of complement regulators to the cell membrane. The first linker to be described is APT070 (Mirococept), which is currently being tested in a clinical trial for prevention of I/R injury in allografts[175–177]. A similar approach using a polyethylene glycol (PEG)-phospholipid linker to bind complement regulators is also under development[178]. In a xenogeneic model in which porcine blood was incubated with human endothelial cells, a factor-H-binding peptide was tested together with an antithrombotic enzyme (apyrase). Despite strong xenogeneic complement-mediated damage to the endothelial cells, this combination of inhibitors protected the cells from complement attack[178]. Taken together, these findings indicate that targeted complement modulation represents a promising future avenue for improving xenograft and allograft survival and transplantation outcomes.

Conclusions

During the lifespan of a transplanted kidney, the functional tissue is gradually damaged to varying degrees by inflammatory and immunological processes. The complement system is as an important contributor to these adverse reactions. Activation of complement is involved in the pathophysiology of many diseases that result in uraemia and may cause deterioration of kidney grafts. Complement is also activated in kidney donors, particularly after brain or cardiac death, resulting in organ damage that affects the quality of the graft and subsequent transplantation outcomes. This complement-driven deterioration of the graft continues during procurement and preservation.

The main mechanism that triggers complement activation in all of these phases of the transplantation process is ischaemia, which results in phenotypic changes to parenchymal and endothelial cells that are recognized by the complement system, and the most damaging event that occurs during the ischaemic period is reperfusion of the graft with blood. Complement has an important role in I/R injury, the severity of which is strongly associated

with the degree and length of the ischaemic period. Complement activity post-transplantation also has a role in tissue damage, particularly during acute and chronic ABMR, which can result in late-stage glomerulopathy and recurrence of kidney disease in the graft.

Owing to the importance of complement activation in allograft injury and its potential impact on transplant outcomes, complement therapeutics are currently being developed as complementary therapies to SOC treatment in clinical transplantation. Although the initial results from early clinical trials are promising, inconsistencies in the effect of complement inhibitors on delayed graft function and ABMR clearly reflect the complexity of allograft injury and the involvement of complement-independent mechanisms. Future well-designed clinical trials with long surveillance are warranted to further evaluate complement interventions in transplantation.

Published online: 26 October 2018

1. Rana, A. et al. Survival outcomes following pediatric liver transplantation (Pedi-SOFT) score: a novel predictive index. *Am. J. Transplant.* **15**, 1855–1863 (2015).
2. Vautmans, H. & Jakovčić, I. Organ donation and transplant in the EU – progress but much more to do. *European Commision* http://ec.europa.eu/health/newsletter/183/focus_newsletter_en.htm (2016).
3. Colvin, R. B. & Smith, R. N. Antibody-mediated organ-allograft rejection. *Nat. Rev. Immunol.* **5**, 807–817 (2005).
4. Ekberg, H. et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N. Engl. J. Med.* **357**, 2562–2575 (2007).
5. Halloran, P. F. et al. Disappearance of T cell-mediated rejection despite continued antibody-mediated rejection in late kidney transplant recipients. *J. Am. Soc. Nephrol.* **26**, 1711–1720 (2015).
6. Halloran, P. F., Famulski, K. S. & Reeve, J. Molecular assessment of disease states in kidney transplant biopsy samples. *Nat. Rev. Nephrol.* **12**, 534–548 (2016).
7. D'Alessandro, A. M. et al. Living unrelated renal donation: the University of Wisconsin experience. *Surgery* **124**, 604–610; discussion 610–611 (1998).
8. Terasaki, P. I., Cecka, J. M., Gjertson, D. W. & Takemoto, S. High survival rates of kidney transplants from spousal and living unrelated donors. *N. Engl. J. Med.* **333**, 333–336 (1995).
9. Voiculescu, A. et al. Kidney transplantation from related and unrelated living donors in a single German centre. *Nephrol. Dial. Transplant.* **18**, 418–425 (2003).
10. Yarlagadda, S. G., Coca, S. G., Formica, R. N., Poggio, E. D. & Parikh, C. R. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol. Dial. Transplant.* **24**, 1039–1047 (2009).
11. Farrar, C. A., Kupiec-Weglinski, J. W. & Sacks, S. H. The innate immune system and transplantation. *Cold Spring Harb. Perspect. Med.* **3**, a015479 (2013).
12. Baldwin, W. M., Ota, H. & Rodriguez, E. R. Complement in transplant rejection: diagnostic and mechanistic considerations. *Springer Semin. Immunopathol.* **25**, 181–197 (2003).
13. Damman, J. et al. Targeting complement activation in brain-dead donors improves renal function after transplantation. *Transpl. Immunol.* **24**, 233–237 (2011).
14. Lin, T., Zhou, W. & Sacks, S. H. The role of complement and Toll-like receptors in organ transplantation. *Transpl. Int.* **20**, 481–489 (2007).
15. Sacks, S. H. & Zhou, W. The role of complement in the early immune response to transplantation. *Nat. Rev. Immunol.* **12**, 431–442 (2012).
16. Cravedi, P. & Heeger, P. S. Complement as a multifaceted modulator of kidney transplant injury. *J. Clin. Invest.* **124**, 2348–2354 (2014).
17. Fuquay, R. et al. Renal ischemia-reperfusion injury amplifies the humoral immune response. *J. Am. Soc. Nephrol.* **24**, 1063–1072 (2013).
18. Wu, W. K., Famure, O., Li, Y. & Kim, S. J. Delayed graft function and the risk of acute rejection in the modern era of kidney transplantation. *Kidney Int.* **88**, 851–858 (2015).
19. Mizuno, M., Suzuki, Y. & Ito, Y. Complement regulation and kidney diseases: recent knowledge of the double-edged roles of complement activation in nephrology. *Clin. Exp. Nephrol.* **22**, 3–14 (2018).
20. Reis, E. S. et al. Therapeutic C3 inhibitor Cp40 abrogates complement activation induced by modern hemodialysis filters. *Immunobiology* **220**, 476–482 (2015).
21. Ekdahl, K. N., Soveri, I., Hilborn, J., Fellstrom, B. & Nilsson, B. Cardiovascular disease in haemodialysis: role of the intravascular innate immune system. *Nat. Rev. Nephrol.* **13**, 285–296 (2017).
22. Mares, J. et al. Proteomic profiling of blood-dialyzer interactome reveals involvement of lectin complement pathway in hemodialysis-induced inflammatory response. *Proteomics Clin. Appl.* **4**, 829–838 (2010).
23. Huang, Z., Gao, D., Letteri, J. J. & Clark, W. R. Blood-membrane interactions during dialysis. *Semin. Dial.* **22**, 623–628 (2009).
24. Nilsson, B., Ekdahl, K. N., Mollnes, T. E. & Lambris, J. D. The role of complement in biomaterial-induced inflammation. *Mol. Immunol.* **44**, 82–94 (2007).
25. Andersson, J., Ekdahl, K. N., Larsson, R., Nilsson, U. R. & Nilsson, B. C3 adsorbed to a polymer surface can form an initiating alternative pathway convertase. *J. Immunol.* **168**, 5786–5791 (2002).
26. Tengvall, P., Askendal, A. & Lundström, I. Complement activation by IgG immobilized on methylated silicon. *J. Biomed. Mater. Res.* **31**, 305–312 (1996).
27. Van Biesen, W., Veys, N., Vanholder, R. & Lameire, N. The impact of the pre-transplant renal replacement modality on outcome after cadaveric kidney transplantation: the ghent experience. *Contrib. Nephrol.* **150**, 254–258 (2006).
28. Fehrman-Ekholm, I., Elinder, C. G., Stenbeck, M., Tydén, G. & Groth, C. G. Kidney donors live longer. *Transplantation* **64**, 976–978 (1997).
29. Ibrahim, H. N. et al. Long-term consequences of kidney donation. *N. Engl. J. Med.* **360**, 459–469 (2009).
30. Kiberd, B. A. & Tennankore, K. K. Lifetime risks of kidney donation: a medical decision analysis. *BMJ Open* **7**, e016490 (2017).
31. Damman, J. et al. Hypoxia and complement-and-coagulation pathways in the deceased organ donor as the major target for intervention to improve renal allograft outcome. *Transplantation* **99**, 1293–1300 (2015).
32. Blogowski, W. et al. Clinical analysis of perioperative complement activity during ischemia/reperfusion injury following renal transplantation. *Clin. J. Am. Soc. Nephrol.* **7**, 1843–1851 (2012).
33. Damman, J. et al. Systemic complement activation in deceased donors is associated with acute rejection after renal transplantation in the recipient. *Transplantation* **92**, 163–169 (2011).
34. Burk, A.-M. et al. Early complementopathy after multiple injuries in humans. *Shock* **37**, 348–354 (2012).
35. Halbgebauer, R. et al. Hemorrhagic shock drives glycocalyx, barrier and organ dysfunction early after polytrauma. *J. Crit. Care* **44**, 229–237 (2017).
36. Huber-Lang, M., Lambris, J. D. & Ward, P. A. Innate immune responses to trauma. *Nat. Immunol.* **19**, 327–341 (2018).
37. van Griensven, M. et al. Protective effects of the complement inhibitor compstatin CP40 in hemorrhagic shock. *Shock* https://doi.org/10.1097/SHK.0000000000001127 (2018).
38. Brown, K. M. et al. Influence of donor C3 allotype on late renal-transplantation outcome. *N. Engl. J. Med.* **354**, 2014–2023 (2006).
39. Damman, J. et al. Association of complement C3 gene variants with renal transplant outcome of deceased cardiac dead donor kidneys. *Am. J. Transplant.* **12**, 660–668 (2012).
40. Sim, E. & Sim, R. B. Enzymic assay of C3b receptor on intact cells and solubilized cells. *Biochem. J.* **210**, 567–576 (1983).
41. Denk, S. et al. Complement C5a functions as a master switch for the pH balance in neutrophils exerting fundamental immunometabolic effects. *J. Immunol.* **198**, 4846–4854 (2017).
42. Farrar, C. A. et al. Collectin-11 detects stress-induced L-fucose pattern to trigger renal epithelial injury. *J. Clin. Invest.* **126**, 1911–1925 (2016).
43. Kolářová, H., Ambrůzová, B., Svihálková Šindlerová, L., Klinke, A. & Kubala, L. Modulation of endothelial glycocalyx structure under inflammatory conditions. *Mediators Inflamm.* **2014**, 694312–694317 (2014).
44. Sieve, I., Münster-Kühnel, A. K. & Hilfiker-Kleiner, D. Regulation and function of endothelial glycocalyx layer in vascular diseases. *Vascul. Pharmacol.* **100**, 26–33 (2018).
45. Yang, G. et al. Novel mechanisms of endothelial dysfunction in diabetes. *J. Cardiovasc. Dis. Res.* **1**, 59–63 (2010).
46. Nguyen, H. X., Galvan, M. D. & Anderson, A. J. Characterization of early and terminal complement proteins associated with polymorphonuclear leukocytes in vitro and in vivo after spinal cord injury. *J. Neuroinflamm.* **5**, 26 (2008).
47. Triantafilou, K., Hughes, T. R., Triantafilou, M. & Morgan, B. P. The complement membrane attack complex triggers intracellular Ca^{2+} fluxes leading to NLRP3 inflammasome activation. *J. Cell. Sci.* **126**, 2903–2913 (2013).
48. Danobeitia, J. et al. Complement blockade prevents delayed graft function in a non-human primate model of kidney allo-transplantation [abstract]. *Am. J Transplant.* **13** (Suppl. 5), 119 (2013).
49. Mathern, D. R. & Heeger, P. S. Molecules great and small: the complement system. *Clin. J. Am. Soc. Nephrol.* **10**, 1636–1650 (2015).
50. Pratt, J. R., Basheer, S. A. & Sacks, S. H. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat. Med.* **8**, 582–587 (2002).
51. Farrar, C. A., Zhou, W., Lin, T. & Sacks, S. H. Local extravascular pool of C3 is a determinant of postischemic acute renal failure. *FASEB J.* **20**, 217–226 (2006).
52. Damman, J. et al. Local renal complement C3 induction by donor brain death is associated with reduced renal allograft function after transplantation. *Nephrol. Dial. Transplant.* **26**, 2345–2354 (2011).
53. Siedlecki, A., Irish, W. & Brennan, D. C. Delayed graft function in the kidney transplant. *Am. J. Transplant.* **11**, 2279–2296 (2011).
54. Kapitsinou, P. P. & Haase, V. H. Molecular mechanisms of ischemic preconditioning in the kidney. *Am. J. Physiol. Renal Physiol.* **309**, F821–F834 (2015).
55. de Vries, D. K. et al. Acute but transient release of terminal complement complex after reperfusion in clinical kidney transplantation. *Transplantation* **95**, 816–820 (2013).
56. Castellano, G. et al. Complement modulation of anti-aging factor klotho in ischemia/reperfusion injury and delayed graft function. *Am. J. Transplant.* **16**, 325–333 (2015).
57. Delpech, P.-O. et al. Inhibition of complement improves graft outcome in a pig model of kidney autotransplantation. *J. Transl Med.* **14**, 701–713 (2016).
58. Thurman, J. M. et al. Treatment with an inhibitory monoclonal antibody to mouse factor B protects mice from induction of apoptosis and renal ischemia/reperfusion injury. *J. Am. Soc. Nephrol.* **17**, 707–715 (2006).
59. Asgari, E. et al. Mannan-binding lectin-associated serine protease 2 is critical for the development of renal ischemia reperfusion injury and mediates tissue injury in the absence of complement C4. *FASEB J.* **28**, 3996–4003 (2014).

60. Walsh, M. C. et al. Mannose-binding lectin is a regulator of inflammation that accompanies myocardial ischemia and reperfusion injury. *J. Immunol.* **175**, 541–546 (2005).
61. Orsini, F. et al. Mannan binding lectin-associated serine protease-2 (MASP-2) critically contributes to post-ischemic brain injury independent of MASP-1. *J. Neuroinflamm.* **13**, 213 (2016).
62. Einecke, G. et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am. J. Transplant.* **9**, 2520–2531 (2009).
63. Haas, M. et al. Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am. J. Transplant.* **14**, 272–283 (2014).
64. Wang, H., Ricklin, D. & Lambris, J. D. Complement-activation fragment C4a mediates effector functions by binding as untethered agonist to protease-activated receptors 1 and 4. *Proc. Natl Acad. Sci. USA* **114**, 10948–10953 (2017).
65. Laumonnier, Y., Karsten, C. M. & Köhl, J. Novel insights into the expression pattern of anaphylatoxin receptors in mice and men. *Mol. Immunol.* **89**, 44–58 (2017).
66. Valenzuela, N. M., Mulder, A. & Reed, E. F. HLA class I antibodies trigger increased adherence of monocytes to endothelial cells by eliciting an increase in endothelial P-selectin and, depending on subclass, by engaging FcγRs. *J. Immunol.* **190**, 6635–6650 (2013).
67. Tedesco, F. et al. The cytolytically inactive terminal complement component complex activates endothelial cells to express adhesion molecules and tissue factor procoagulant activity. *J. Exp. Med.* **185**, 1619–1627 (1997).
68. Brunn, G. J. Differential regulation of endothelial cell activation by complement and interleukin 1. *Circ. Res.* **98**, 793–800 (2006).
69. Foreman, K. E. et al. C5a-induced expression of P-selectin in endothelial cells. *J. Clin. Invest.* **94**, 1147–1155 (1994).
70. Ikeda, K. et al. C5a induces tissue factor activity on endothelial cells. *Thromb. Haemost.* **77**, 394–398 (1997).
71. Jane-wit, D. et al. Alloantibody and complement promote T cell-mediated cardiac allograft vasculopathy through noncanonical nuclear factor-B signaling in endothelial cells. *Circulation* **128**, 2504–2516 (2013).
72. Stegall, M. D., Chedid, M. F. & Cornell, L. D. The role of complement in antibody-mediated rejection in kidney transplantation. *Nat. Rev. Nephrol.* **8**, 670–678 (2012).
73. Panda, S. & Ding, J. L. Natural antibodies bridge innate and adaptive immunity. *J. Immunol.* **194**, 13–20 (2015).
74. Garcia de Mattos Barbosa, M., Cascalho, M. & Platt, J. L. Accommodation in ABO-incompatible organ transplants. *Xenotransplantation* **25**, e12418 (2018).
75. Sheil, A. G., Stewart, J. H., Tiller, D. J. & May, J. ABO blood group incompatibility in renal transplantation. *Transplantation* **8**, 299–300 (1969).
76. Ugurlar, D. et al. Structures of C1-IgG1 provide insights into how danger pattern recognition activates complement. *Science* **359**, 794–797 (2018).
77. De Clippel, D. et al. Screening for HLA antibodies in plateletpheresis donors with a history of transfusion or pregnancy. *Transfusion* **54**, 3036–3042 (2014).
78. Saadi, S., Takahashi, T., Holzknecht, R. A. & Platt, J. L. Pathways to acute humoral rejection. *Am. J. Pathol.* **164**, 1073–1080 (2004).
79. Valenzuela, N. M., McNamara, J. T. & Reed, E. F. Antibody-mediated graft injury: complement-dependent and complement-independent mechanisms. *Curr. Opin. Organ Transplant.* **19**, 33–40 (2014).
80. Dahlbäck, B. & Hildebrand, B. Degradation of human complement component C4b in the presence of the C4b-binding protein-protein S complex. *Biochem. J.* **209**, 857–863 (1983).
81. Hamer, R. et al. Human leukocyte antigen-specific antibodies and gamma-interferon stimulate human microvascular and glomerular endothelial cells to produce complement factor C4. *Transplantation* **93**, 867–873 (2012).
82. Loupy, A. et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N. Engl. J. Med.* **369**, 1215–1226 (2013).
83. Sicard, A. et al. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. *J. Am. Soc. Nephrol.* **26**, 457–467 (2015).
84. Lefaucheur, C. et al. Complement-activating anti-HLA antibodies in kidney transplantation: allograft gene expression profiling and response to treatment. *J. Am. Soc. Nephrol.* **29**, 620–635 (2018).
85. Zipfel, P. F. et al. The role of complement in C3 glomerulopathy. *Mol. Immunol.* **67**, 21–30 (2015).
86. Sethi, S. & Fervenza, F. C. Membranoproliferative glomerulonephritis—a new look at an old entity. *N. Engl. J. Med.* **366**, 1119–1131 (2012).
87. Le Quintrec, M. et al. Complement genes strongly predict recurrence and graft outcome in adult renal transplant recipients with atypical hemolytic and uremic syndrome. *Am. J. Transplant.* **13**, 663–675 (2013).
88. Salvadori, M. & Bertoni, E. Complement related kidney diseases: recurrence after transplantation. *World J. Transplant.* **6**, 632–645 (2016).
89. Poppelaars, F. et al. C1-inhibitor treatment decreases renal injury in an established brain-dead rat model. *Transplantation* **102**, 79–87 (2017).
90. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02435732 (2017).
91. Lewis, A. G., Kohl, G., Ma, Q., Devarajan, P. & Kohl, J. Pharmacological targeting of C5a receptors during organ preservation improves kidney graft survival. *Clin. Exp. Immunol.* **153**, 117–126 (2008).
92. Rich, M. C. et al. Site-targeted complement inhibition by a complement receptor 2-conjugated inhibitor (mTT30) ameliorates post-injury neuropathology in mouse brains. *Neurosci. Lett.* **617**, 188–194 (2016).
93. Ruseva, M. M., Ramaglia, V., Morgan, B. P. & Harris, C. L. An anticomplement agent that homes to the damaged brain and promotes recovery after traumatic brain injury in mice. *Proc. Natl Acad. Sci. USA* **112**, 14319–14324 (2015).
94. Yu, Z. X. et al. Targeting complement pathways during cold ischemia and reperfusion prevents delayed graft function. *Am. J. Transplant.* **16**, 2589–2597 (2016).
95. Emlen, W., Li, W. & Kirschfink, M. Therapeutic complement inhibition: new developments. *Semin. Thromb. Hemost.* **36**, 660–668 (2010).
96. Ricklin, D., Mastellos, D. C., Reis, E. S. & Lambris, J. D. The renaissance of complement therapeutics. *Nat. Rev. Nephrol.* **14**, 26–47 (2018).
97. Parker, C. Eculizumab for paroxysmal nocturnal haemoglobinuria. *Lancet* **373**, 759–767 (2009).
98. Zuber, J. et al. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nat. Rev. Nephrol.* **8**, 643–657 (2012).
99. Howard, J. F. et al. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. *Muscle Nerve* **48**, 76–84 (2013).
100. Burbach, M. et al. Report of the inefficacy of eculizumab in two cases of severe antibody-mediated rejection of renal grafts. *Transplantation* **98**, 1056–1059 (2014).
101. Cornell, L. D., Schinstock, C. A., Gandhi, M. J., Kremers, W. K. & Stegall, M. D. Positive crossmatch kidney transplant recipients treated with eculizumab: outcomes beyond 1 year. *Am. J. Transplant.* **15**, 1293–1302 (2015).
102. González-Roncero, F. et al. Eculizumab treatment of acute antibody-mediated rejection in renal transplantation: case reports. *Transplant. Proc.* **44**, 2690–2694 (2012).
103. Locke, J. E. et al. The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am. J. Transplant.* **9**, 231–235 (2009).
104. Stegall, M. D. et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am. J. Transplant.* **11**, 2405–2413 (2011).
105. Yelken, B. et al. Eculizumab for treatment of refractory antibody-mediated rejection in kidney transplant patients: a single-center experience. *Transplant. Proc.* **47**, 1754–1759 (2015).
106. Orandi, B. J. et al. Eculizumab and splenectomy as salvage therapy for severe antibody-mediated rejection after HLA-incompatible kidney transplantation. *Transplantation* **98**, 857–863 (2014).
107. Biglarnia, A.-R. et al. Prompt reversal of a severe complement activation by eculizumab in a patient undergoing intentional ABO-incompatible pancreas and kidney transplantation. *Transplant Int.* **24**, e61–e66 (2011).
108. West-Thielke, P. et al. Eculizumab for prevention of antibody-mediated rejection in blood group-incompatible renal transplantation. *Transplant. Proc.* **50**, 66–69 (2018).
109. Bentall, A. et al. Antibody-mediated rejection despite inhibition of terminal complement. *Transpl. Int.* **27**, 1235–1243 (2014).
110. Alexion. Alexion provides update on phase 2 clinical trial with eculizumab in antibody mediated rejection (AMR) in living-donor kidney transplant recipients. *AlexionPharma* https://news.alexionpharma.com/press-release/company-news/alexion-provides-update-phase-2-clinical-trial-eculizumab-antibody-mediat (2015).
111. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01399593 (2018).
112. Harder, M. J. et al. Incomplete inhibition by eculizumab: mechanistic evidence for residual C5 activity during strong complement activation. *Blood* **129**, 970–980 (2017).
113. Kirschfink, M. C1-inhibitor and transplantation. *Immunobiology* **205**, 534–541 (2002).
114. Tillou, X. et al. Recombinant human C1-inhibitor prevents acute antibody-mediated rejection in alloimmunized baboons. *Kidney Int.* **78**, 152–159 (2010).
115. Vo, A. A. et al. A phase I/II placebo-controlled trial of C1-inhibitor for prevention of antibody-mediated rejection in HLA sensitized patients. *Transplantation* **99**, 299–308 (2015).
116. Viglietti, D. et al. C1 inhibitor in acute antibody-mediated rejection nonresponsive to conventional therapy in kidney transplant recipients: a pilot study. *Am. J. Transplant.* **16**, 1596–1603 (2016).
117. Montgomery, R. A. 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.* **16**, 3468–3478 (2016).
118. Halloran, P. F., Reeve, J. P., Pereira, A. B., Hidalgo, L. G. & Famulski, K. S. Antibody-mediated rejection, T cell–mediated rejection, and the injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies. *Kidney Int.* **85**, 258–264 (2014).
119. Eskandary, F. et al. Anti-C1s monoclonal antibody BIVV009 in late antibody-mediated kidney allograft rejection-results from a first-in-patient phase 1 trial. *Am. J. Transplant.* **8**, 670–926 (2017).
120. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03347396 (2018).
121. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03316521 (2018).
122. Mastellos, D. C. et al. Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention. *Eur. J. Clin. Invest.* **45**, 423–440 (2015).
123. Qu, H. et al. New analogs of the clinical complement inhibitor compstatin with subnanomolar affinity and enhanced pharmacokinetic properties. *Immunobiology* **218**, 496–505 (2013).
124. Pawel-Rammingen, von, U. & Björck, L. IdeS and SpeB: immunoglobulin-degrading cysteine proteinases of Streptococcus pyogenes. *Curr. Opin. Microbiol.* **6**, 50–55 (2003).
125. Brezski, R. J. et al. Tumor-associated and microbial proteases compromise host IgG effector functions by a single cleavage proximal to the hinge. *Proc. Natl Acad. Sci. USA* **106**, 17864–17869 (2009).
126. Jordan, S. C. et al. IgG endopeptidase in highly sensitized patients undergoing transplantation. *N. Engl. J. Med.* **377**, 442–453 (2017).
127. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02224820 (2017).
128. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02426684 (2017).
129. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02475551 (2018).
130. Platt, J. L. et al. Transplantation of discordant xenografts: a review of progress. *Immunol. Today* **11**, 450–456 (1990).
131. Park, W. D. et al. Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am. J. Transplant.* **3**, 952–960 (2003).
132. Zhong, S. et al. Complement inhibition enables renal allograft accommodation and long-term engraftment in presensitized nonhuman primates. *Am. J. Transplant.* **11**, 2057–2066 (2011).

133. Narayanan, K., Jendrisak, M. D., Phelan, D. L. & Mohanakumar, T. HLA class I antibody mediated accommodation of endothelial cells via the activation of PI3K/cAMP dependent PKA pathway. *Transpl. Immunol.* **15**, 187–197 (2006).
134. Dijke, E. I. et al. B cells in transplantation. *J. Heart Lung Transplant.* **35**, 704–710 (2016).
135. Chen Song, S. et al. Complement inhibition enables renal allograft accommodation and long-term engraftment in presensitized nonhuman primates. *Am. J. Transplant.* **11**, 2057–2066 (2011).
136. Dehoux, J.-P. & Gianello, P. Accommodation and antibodies. *Transpl. Immunol.* **21**, 106–110 (2009).
137. Benson, B. A., Vercellotti, G. M. & Dalmasso, A. P. IL-4 and IL-13 induce protection from complement and melittin in endothelial cells despite initial loss of cytoplasmic proteins: membrane resealing impairs quantifying cytotoxicity with the lactate dehydrogenase permeability assay. *Xenotransplantation* **22**, 295–301 (2015).
138. Suhr, B. D., Black, S. M., Guzman-Paz, M., Matas, A. J. & Dalmasso, A. P. Inhibition of the membrane attack complex of complement for induction of accommodation in the hamster-to-rat heart transplant model. *Xenotransplantation* **14**, 572–579 (2007).
139. Tan, C. D. et al. Correlation of donor-specific antibodies, complement and its regulators with graft dysfunction in cardiac antibody-mediated rejection. *Am. J. Transplant.* **9**, 2075–2084 (2009).
140. Griesemer, A. D. et al. Upregulation of CD59: potential mechanism of accommodation in a large animal model. *Transplantation* **87**, 1308–1317 (2009).
141. Platt, J. L., Kaufman, C. L., Garcia de Mattos Barbosa, M. & Cascalho, M. Accommodation and related conditions in vascularized composite allografts. *Curr. Opin. Organ Transplant.* **22**, 470–476 (2017).
142. Bannett, A. D., McAlack, R. F., Morris, M., Chopek, M. W. & Platt, J. L. ABO incompatible renal transplantation: a qualitative analysis of native endothelial tissue ABO antigens after transplantation. *Transplant. Proc.* **21**, 783–785 (1989).
143. Chopek, M. W., Simmons, R. L. & Platt, J. L. ABO-incompatible kidney transplantation: initial immunopathologic evaluation. *Transplant. Proc.***19**, 4553–4557 (1987).
144. Wang, H. et al. Inhibition of terminal complement components in presensitized transplant recipients prevents antibody-mediated rejection leading to long-term graft survival and accommodation. *J. Immunol.* **179**, 4451–4463 (2007).
145. Wang, H. et al. Prevention of acute vascular rejection by a functionally blocking anti-C5 monoclonal antibody combined with cyclosporine. *Transplantation* **79**, 1121–1127 (2005).
146. Vogel, C.-W. & Fritzinger, D. C. Cobra venom factor: structure, function, and humanization for therapeutic complement depletion. *Toxicon* **56**, 1198–1222 (2010).
147. Montero, R. M., Sacks, S. H. & Smith, R. A. Complement-here, there and everywhere, but what about the transplanted organ? *Semin. Immunol.* **28**, 250–259 (2016).
148. Pepys, M. B. Role of complement in induction of antibody production in vivo. Effect of cobra factor and other C3-reactive agents on thymus-dependent and thymus-independent antibody responses. *J. Exp. Med.* **140**, 126–145 (1974).
149. Carroll, M. C. Complement and humoral immunity. *Vaccine* **26** (Suppl. 8), I28–133 (2008).
150. Heyman, B., Wiersma, E. J. & Kinoshita, T. In vivo inhibition of the antibody response by a complement receptor-specific monoclonal antibody. *J. Exp. Med.* **172**, 665–668 (1990).
151. Carter, R. H. & Fearon, D. T. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* **256**, 105–107 (1992).
152. Prodeus, A. P. et al. A critical role for complement in maintenance of self-tolerance. *Immunity* **9**, 721–731 (1998).
153. Sacks, S., Lee, Q., Wong, W. & Zhou, W. The role of complement in regulating the alloresponse. *Curr. Opin. Organ Transplant* **14**, 10–15 (2009).
154. Heeger, P. S. & Kemper, C. Novel roles of complement in T effector cell regulation. *Immunobiology* **217**, 216–224 (2012).
155. Arbore, G. et al. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4+ T cells. *Science* **352**, aad1210 (2016).
156. Quell, K. M. et al. Monitoring C3aR expression using a floxed tdTomato-C3aR reporter knock-in mouse. *J. Immunol.* **199**, 688–706 (2017).
157. Karsten, C. M. et al. Monitoring C5aR2 expression using a floxed tdTomato-C5aR2 knock-in mouse. *J. Immunol.* **199**, 3234–3248 (2017).
158. Kwan, W.-H., van der Touw, W., Paz-Artal, E., Li, M. O. & Heeger, P. S. Signaling through C5a receptor and C3a receptor diminishes function of murine natural regulatory T cells. *J. Exp. Med.* **210**, 257–268 (2013).
159. Strainic, M. G. et al. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. *Immunity* **28**, 425–435 (2008).
160. Le Friec, G., Köhl, J. & Kemper, C. A complement a day keeps the Fox(p3) away. *Nat. Immunol.* **14**, 110–112 (2013).
161. Ellinghaus, U. et al. Dysregulated CD46 shedding interferes with Th1-contraction in systemic lupus erythematosus. *Eur. J. Immunol.* **47**, 1200–1210 (2017).
162. Strainic, M. G., Shevach, E. M., An, F., Lin, F. & Medof, M. E. Absence of signaling into $CD4^+$ cells via C3aR and C5aR enables autoinductive TGF-β1 signaling and induction of $Foxp3^+$ regulatory T cells. *Nat. Immunol.* **14**, 162–171 (2013).
163. van der Touw, W. et al. Cutting edge: receptors for C3a and C5a modulate stability of alloantigen-reactive induced regulatory T cells. *J. Immunol.* **190**, 5921–5925 (2013).
164. Braza, F., Durand, M., Degauque, N. & Brouard, S. Regulatory T cells in kidney transplantation: new directions? *Am. J. Transplant.* **15**, 2288–2300 (2015).
165. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02129881 (2014).
166. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02088931 (2016).
167. Jiménez-Reinoso, A. et al. Human plasma C3 is essential for the development of memory B, but not T, lymphocytes. *J. Allergy Clin. Immunol.* **141**, 1151–1154.e14 (2017).
168. Gueler, F. et al. Complement 5a receptor inhibition improves renal allograft survival. *J. Am. Soc. Nephrol.* **19**, 2302–2312 (2008).
169. Li, Q. et al. Deficiency of C5aR prolongs renal allograft survival. *J. Am. Soc. Nephrol.* **21**, 1344–1353 (2010).
170. Farrar, C. A., Zhou, W. & Sacks, S. H. Role of the lectin complement pathway in kidney transplantation. *Immunobiology* **221**, 1068–1072 (2016).
171. Wijkstrom, M. et al. Islet allograft survival in nonhuman primates immunosuppressed with basiliximab, RAD, and FTY7201. *Transplantation* **77**, 827–835 (2004).
172. Atkinson, J. P., Oglesby, T. J., White, D., Adams, E. A. & Liszewski, M. K. Separation of self from non-self in the complement system: a role for membrane cofactor protein and decay accelerating factor. *Clin. Exp. Immunol.* **86** (Suppl. 1), 27–30 (1991).
173. Cooper, D. K. C., Ekser, B., Ramsoondar, J., Phelps, C. & Ayares, D. The role of genetically engineered pigs in xenotransplantation research. *J. Pathol.* **238**, 288–299 (2016).
174. Yamanaka, K. et al. Depression of complement regulatory factors in rat and human renal grafts is associated with the progress of acute T-cell mediated rejection. *PLOS ONE* **11**, e0148881 (2016).
175. Souza, D. G., Esser, D., Bradford, R., Vieira, A. T. & Teixeira, M. M. APT070 (Mirococept), a membrane-localised complement inhibitor, inhibits inflammatory responses that follow intestinal ischaemia and reperfusion injury. *Br. J. Pharmacol.* **145**, 1027–1034 (2005).
176. Patel, H. Therapeutic strategy with a membrane-localizing complement regulator to increase the number of usable donor organs after prolonged cold storage. *J. Am. Soc. Nephrol.* **17**, 1102–1111 (2006).
177. Kassimatis, T. 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* **18**, 2279–2211 (2017).
178. Nilsson, P. H. et al. Autoregulation of thromboinflammation on biomaterial surfaces by a multicomponent therapeutic coating. *Biomaterials* **34**, 985–994 (2013).
179. Hinglais, N. et al. Immunohistochemical study of the C5b-9 complex of complement in human kidneys. *Kidney Int.* **30**, 399–410 (1986).
180. Okada, M. et al. Immunohistochemical localization of C3d fragment of complement and S-protein (vitronectin) in normal and diseased human kidneys: association with the C5b-9 complex and vitronectin receptor. *Virchows Arch. A Pathol. Anat. Histopathol.* **422**, 367–373 (1993).
181. Sacks, S. H., Zhou, W., Pani, A., Campbell, R. D. & Martin, J. Complement C3 gene expression and regulation in human glomerular epithelial cells. *Immunology* **79**, 348–354 (1993).
182. Mekori, Y. A., Steiner, P., Farkash, R., Moalem, T. & Klajman, A. Deposits of immunoglobulins and C3 in the walls of human renal arteries. *Clin. Exp. Immunol.* **43**, 254–259 (1981).
183. Feucht, H. E. et al. Detection of both isotypes of complement C4, C4A and C4B, in normal human glomeruli. *Kidney Int.* **30**, 932–936 (1986).
184. Zwirner, J., Felber, E., Herzog, V., Riethmüller, G. & Feucht, H. E. Classical pathway of complement activation in normal and diseased human glomeruli. *Kidney Int.* **36**, 1069–1077 (1989).
185. Song, D., Zhou, W., Sheerin, S. H. & Sacks, S. H. Compartmental localization of complement component transcripts in the normal human kidney. *Nephron* **78**, 15–22 (1998).
186. Cosio, F. G., Sedmak, D. D., Mahan, J. D. & Nahman, N. S. Localization of decay accelerating factor in normal and diseased kidneys. *Kidney Int.* **36**, 100–107 (1989).
187. Nakanishi, I. et al. Identification and characterization of membrane cofactor protein (CD46) in the human kidneys. *Eur. J. Immunol.* **24**, 1529–1535 (1994).
188. Endoh, M. et al. Immunohistochemical demonstration of membrane cofactor protein (MCP) of complement in normal and diseased kidney tissues. *Clin. Exp. Immunol.* **94**, 182–188 (1993).
189. Ichida, S., Yuzawa, Y., Okada, H., Yoshioka, K. & Matsuo, S. Localization of the complement regulatory proteins in the normal human kidney. *Kidney Int.* **46**, 89–96 (1994).
190. Jokiranta, T. S. et al. Binding of complement factor H to endothelial cells is mediated by the carboxy-terminal glycosaminoglycan binding site. *Am. J. Pathol.* **167**, 1173–1181 (2005).
191. Lesher, A. M. & Song, W.-C. Review: complement and its regulatory proteins in kidney diseases. *Nephrology (Carlton)* **15**, 663–675 (2010).
192. Appay, M. D., Kazatchkine, M. D., Levi-Strauss, M., Hinglais, N. & Bariety, J. Expression of CR1 (CD35) mRNA in podocytes from adult and fetal human kidneys. *Kidney Int.* **38**, 289–293 (1990).
193. Fayyazi, A. et al. The C5a receptor is expressed in normal renal proximal tubular but not in normal pulmonary or hepatic epithelial cells. *Immunology* **99**, 38–45 (2000).
194. Zahedi, R. et al. The C5a receptor is expressed by human renal proximal tubular epithelial cells. *Clin. Exp. Immunol.* **121**, 226–233 (2000).
195. Braun, M. C. et al. Renal expression of the C3a receptor and functional responses of primary human proximal tubular epithelial cells. *J. Immunol.* **173**, 4190–4196 (2004).
196. Li, X., Ding, F., Zhang, X., Li, B. & Ding, J. The expression profile of complement components in podocytes. *Int. J. Mol. Sci.* **17**, 471 (2016).
197. Liu, L. et al. C3a, C5a renal expression and their receptors are correlated to severity of IgA nephropathy. *J. Clin. Immunol.* **34**, 224–232 (2014).

Acknowledgements
The authors thank Deborah McClellan for excellent editorial assistance before the manuscript was submitted. The European Community's Seventh Framework Programme under the grant agreement n°602699 (DIREKT) has been a major contributor to the authors' work, which was further supported by grant 2016-2075-5.1 and 2016–04519 from the Swedish Research Council (VR), and by the Deutsche Forschungsgemeinschaft (DFG) grant CRC1149 A01.

Author contributions
All authors researched the data, made substantial contributions to discussions of the content, wrote the text and reviewed or edited the manuscript before submission.

Competing interests
The authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Reviewer information
Nature Reviews Nephrology thanks S. Jordan, D. Ricklin and the other anonymous reviewer(s) for their contribution to the peer review of this work.