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Complement- here, there and everywhere, but what about the transplanted organ?

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Abstract

The part of the innate immune system that communicates and effectively primes the adaptive immune system was termed “complement” by Ehrlich to reflect its complementarity to antibodies having previously been described as “alexine” (i.e protective component of serum) by Buchner and Bordet. It has been established that complement is not solely produced systemically but may have origin in different tissues where it can influence organ specific functions that may affect the outcome of transplanted organs. This review looks at the role of complement in particular to kidney transplantation. We look at current literature to determine whether blockade of the peripheral or central compartments of complement production may prevent ischaemic reperfusion injury or rejection in the transplanted organ.

We also review new therapeutics that have been developed to inhibit components of the complement cascade with varying degrees of success leading to an increase in our understanding of the multiple triggers of this complex system. In addition, we consider whether biomarkers in this field are effective markers of disease or treatment.

Word count 169

Keywords: Complement, Kidney transplantation, Mirococept, Therapeutics

1. Introduction

1.1 What is complement?

The complement system was first recognised as a fundamental part of the innate immune system in the late 1890s. It initiates and maintains host defence by combining recognition molecules, proteolytic enzymes, receptors and foreign or altered internal products. The complement system comprises of 35-40 components and regulators that are soluble or membrane bound and catalyse the breakdown of converting enzyme complexes or convertases that lead to the formation of membrane attack complex (C5b, C6, C7, C8 and C9 (C5b-C9)). Activation of the complement cascade has been described to use three pathways; the classical, lectin and alternative pathways (Fig 1). The classical pathway is triggered by antigen-antibody immune complexes arising from immune surveillance molecules (e.g. IgG, IgM, C-reactive protein) leading to activation of C4, C2 and C3. The lectin pathway is initiated by collectins (e.g. mannose binding lectin, MBL) and ficolins, which bind to carbohydrate ligands on the surface of pathogens. This subsequently leads to the formation of C4b, C2a - the C3 convertase complex similar to the classical pathway. In contrast, the alternative pathway is activated by either spontaneous hydrolysis of C3 or by C3b binding to the activated surface thus amplifying the cascade, using Factors B, D and P to form the alternative C3 convertase C3bBb.

Initiation of the complement cascade results in the deposition of C3 onto the activating surface. Cleavage of C3 forms C3a and C3b with the latter triggering formation of C5 convertase. C5 is cleaved leading to formation of C5b-C9. The anaphylatoxins (C3a and C5a) are potent pro-inflammatory molecules that are chemoattractant and activate leucocytes via C3aR and CD88 (C5aR1). Opsonins (C3b, inactive C3b (iC3b) and degradation product C3d) remain covalently bound to target surfaces facilitating transport and removal of the targeted cells or immune complexes. Formation of C5b-9 in the targeted cell membrane results in direct lysis of the pathogen causing cell activation, injury and death [1]. The complement system is regulated by a number of inhibitors and regulators to prevent local destruction of tissues. CD35 (CR1), CD46 (MCP), CD55 (DAF), C4BP (C4b-binding protein) and Factor H are all members of the same family of complement regulatory proteins that have similar structures with varying number of subunits that are encoded on chromosome 1 and inhibit the functions of the converting enzyme complexes that cleave C3 and C5. In addition, CD59 inhibits the formation of C5b-C9. Complement regulators have a number of functions that are described in Table 1. Dysregulation of the complement regulators has increasingly been described in a number of diseases such as age-related macular degeneration, systemic lupus erythematosus (SLE), spinal cord injuries, atypical haemolytic uraemic syndrome (aHUS), arthritis, autoimmune heart disease and paroxysmal nocturnal hemoglobinuria) [2-7]. Complement has also been described to be important in priming the adaptive immune system whereby C3a and C5a released by local complement activation act as cofactors for stimulation of antigen presentation whilst enhancing activation of naïve alloreactive T cells [8-12].

1.2 Peripheral and central complement production

An intra and extravascular pool of complement has previously been described whereby hepatic synthesis mainly maintains the intravascular pool. In contrast the extravascular pool arises from peripheral or local tissue cellular activity from tissue-resident and migratory cells such as antigen presenting cells (APCs), T cells and

tubuloepithelial cells in the kidney [13, 14]. It has been argued that C3 could be maintained in the central compartment due to its 180kDa size; however it is ill-understood as to whether retention of this large complement molecule in the central compartment is due to size alone [15, 16]. Other circulating complement factors including C2, C4 and Factor B can similarly be generated in tissue-resident cells. Animal studies have previously described the important role of central and peripheral pools of C3 with uncontrolled locally produced complement resulting in effective and rapid complement mediated tissue damage [15]. C3 arising from the transplanted kidney following an ischaemic insult has been described to be dependent on the cold ischaemic time prior to surgery with deposited C3 levels peaking approximately 48 hours following the surgery, though in patients it has been reported that complement activation may begin in the organ donor [17, 18]. This results in significant damage to the tubuloepithelial cells that are susceptible to hypoxic damage. Tubuloepithelial cells are the main source of local complement in the kidney, however all cellular compartments of the kidney are able to synthesise complement, based on in vivo and ex vivo analysis [19]. Improving our understanding in this area and of the inter-individual variations that confer disease susceptibility will allow us to deliver targeted specific personalised medicine to prevent the decline in allograft function [20]. The remainder of this review will focus on the role of complement in kidney transplantation, which is the most common type of solid-organ transplantation performed.

2. Kidney transplantation

The complement system has provided a natural barrier to xenotransplantation for many years, with damage to pig organs arising from exposure to human serum [21, 22]. Studies in allotransplantation have also highlighted the importance of complement in alloantibody-mediated rejection. Subsequently it became apparent that peripheral complement also plays a central role determining how the donor organ responds to the stress of transplantation [23]. Further work has identified how alloimmune priming and cell-mediated rejection are complement-dependent. In this review, we shall focus on the role of complement in several of these functions that are relevant to current clinical practice.

2.1 Ischaemia-reperfusion injury

Ischaemia-reperfusion (IR) injury is thought to be the main contributor to the development of delayed graft function (DGF). DGF occurs in a third of all deceased donor organs rising to 50% in circulatory deceased donors [24]. This has led to an increase in reperfusion machine studies to determine whether the injury sustained may decrease with normothermic or hypothermic perfusion [25, 26]. IR injury arises from a combination of tissue hypoxia, mitochondrial damage, ATP depletion and free oxygen radicals on reperfusion, leading to damage of the endothelium and epithelium. This induces cellular inflammation via toll-like receptors, cytokines, chemokines and complement. Complement activation both initiates and propagates tissue damage not only in kidney transplantation but also in other major transplanted organs such as the heart, lung, liver and pancreas [27-32].

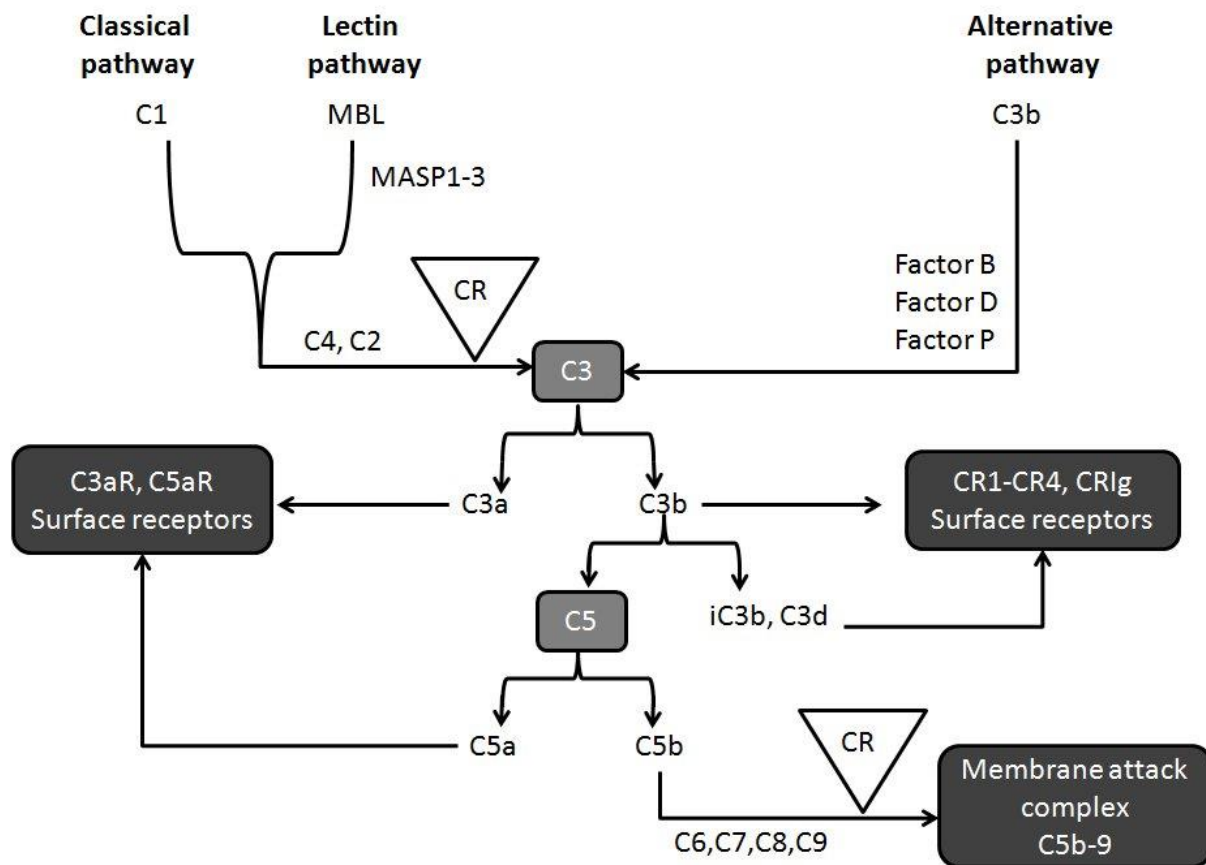


Figure 1. The complement cascades.

The classical pathway is triggered by the C1 complex (C1q, C1r, C1s) binding to antibody or C-reactive protein on the activating surface, leading to the formation of the classical pathway C3 convertase from C4 and C2 (C4bC2a) and to subsequent cleavage of C3. The lectin pathway is activated by collectins (e.g. Mannose binding lectin, MBL) or ficolins that detect carbohydrate residues, IgA or IgM on the activating surface. Complexes formed between the lectin molecule and MBL-associated serine proteases result in the formation of C3 convertase (C4bC2a), as per the classical pathway. In contrast the alternative pathway begins with direct binding of C3b to the activating surface after spontaneous hydrolysis of C3 or following the deposition of C3b by the classical or lectin pathway. In this case, C3b binds factor B followed by cleavage of factor B to form the alternative pathway C3 convertase (C3bBb). All three pathways form enzyme complexes (classical or alternative convertases) that cleave C3 into C3a and C3b and ultimately cleave C5 into C5a and C5b. C5b triggers the terminal pathway forming the membrane attack complex (C5b-C9), which creates a pore in the cells membrane resulting in cell lysis and death. Specific cell surface receptors detect C3a, C5a, iC3b, C3b and C3d. Complement regulators (CR) include: CD35 (CR1), CD46 (MCP), CD55 (DAF), C4BP (C4b-binding protein) and complement Factor H, which function by accelerating decay of the C3 and C5 convertases or by acting as cofactors for the proteolysis of these convertases by Factor I, or have both of these actions; and CD59 (Protectin), which binds C8 and inhibits membrane attack complex formation Table 1 provides further details of CR.

Complement-mediated injury following IR insult mainly arises from C5a and C5b-C9 terminal pathway products. This has been found in studies of complement deficient and depleted mice that are protected against reperfusion damage [30, 31, 33, 34]. C5a acts directly on the C5a receptor on parenchymal cells, as well as stimulating chemotaxis and degranulation of neutrophils [35, 36]. In contrast C3a is reported to have a less significant role in IR injury [37]. It has been suggested that donor-kidney derived C3 is a more effective driver of IR injury than the systemic recipient C3 [15]. The degree of C3b involvement in this process is presently unclear.

We have previously described an increase in the synthesis of C3 in transplanted kidneys to be dependent on the length of cold ischaemia prior to transplantation in a mouse model [15]. A number of other stress-related factors such as interferon- γ (IFN γ), lipopolysaccharide (LPS) and IL-1 may also increase the production of complement generating C5a and C5b-C9 and consequent graft dysfunction. Complement activation on endothelial and epithelial cells in turn can upregulate or mediate the release of pro-inflammatory and fibrotic factors including tumour necrosis factor (TNF), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and collagen causing chronic tissue damage if they remain unregulated [38-40]. The presence of complement factors in human donor kidneys pre-transplantation have previously been reported with poor allograft function 3 years later [19]. The balance of complement production and regulation is cell dependent within the organ affected. It appears that endothelial cells and myocytes are the predominant targets in post ischaemic heart and intestine respectively, whilst proximal tubuloepithelial cells regulate damage within the transplanted kidney [18, 24, 37, 38, 41, 42].

IR injury may also arise from activation of complement receptors. This has been suggested from reports in brain deceased donors whose raised donor CD88 expression on renal tubuloepithelial cells is associated with worse allograft function compared to living donors [43]. In addition, bone marrow chimera studies in mice deficient in C3aR and CD88 on renal tubuloepithelial cells or circulating leucocytes resulted in little IR injury in the kidney [44]. C5b-9 has also been reported to be detected in pre-implantation renal allograft biopsies of human deceased donors with none seen in live donors. In addition, whole genome profiling has discovered a number of highly expressed complement genes in human donor organs with IR thereby supporting the important role of complement in IR injury [19, 45].

2.1.1 Complement activation pathways in IR injury

Each complement pathway uses different triggers that respond to various pathogenic factors such as infection, ischaemia and hypothermia. Initially it was thought that the classical pathway was involved in IR as C4 and natural IgM were shown to be central to IR damage in cardiac and skeletal muscle and later in intestinal tissue [41, 46, 47]. Classical pathway activation is dependent on C1q binding to immunoglobulins, C-reactive protein or other tissue-adherent surveillance molecules; however recent studies have shown IgM to stimulate the lectin pathway directly thus questioning the role of the classical pathway in IR injury.

IR specifically stimulates the lectin pathway where MBL binds to carbohydrate residues on the activating surface to trigger complement mediated damage [46, 48-50]. MBL-associated serine proteases (MASP-1,2 and 3) are proteolytic enzymes that bind to collectins and ficolins to initiate complement activation. MASP-2 is notable in that it is able to mediate cleavage of C3 without C4 [51]. MBL has been described to be internalised by renal proximal tubuloepithelial cells in organ reperfusion whereby they are destroyed by apoptosis in a murine model [52]. Interestingly this cytotoxic effect is independent of complement activation and specific to MBL. Studies have also reported activation of the lectin pathway via binding of IgM with myosin like molecules exposed in tissues with ischaemic injury [53]. Identifying other triggers of the lectin pathway in IR may lead to novel specific therapeutic targets. A recent study demonstrates a role for collectin-11 (CL-11) in renal IR injury, whereby CL-11 produced by the renal tubuloepithelial cell appears to trigger complement activation along the basolateral tubuloepithelial surface [54].

In a study looking at simultaneous kidney pancreas transplantation, low circulating levels of MBL were associated with good allograft outcome [55]. A different study associated low MBL levels with poor renal allograft function and survival in non-HLA sensitised patients [56]. These differences may arise from the detection assay used with different antibodies binding high or low-order oligomers of MBL. High-oligomers of MBL have higher affinity for carbohydrates thereby stimulating the lectin pathway [57]. Poor graft survival has also been associated with high serum levels of ficolin-3, a recognition molecule that activates the lectin pathway, in a study of >500 renal transplant recipients [58].

The alternative pathway involves complement factor B thereby facilitating an increase in C3b available for deposition on cells from the above pattern recognition pathways. Murine studies have shown factor B deficient mice to be protected from IR injury and therefore its presence may significantly amplify the amount of injury mediated by the complement system [59]. Human heart and kidney transplant biopsies have been reported to have products from the lectin and alternative pathway, respectively [10, 18, 60]. Complement is also known to impair vasculature remodelling that is required post transplantation and thus may induce or perpetuate the ischaemic injury on the organ [61].

2.2 Kidney Transplant Rejection

2.2.1 Cell-mediated allograft rejection

Products of complement can be detected in the blood, urine and organ biopsies of patients with cellular cardiac or kidney rejection [62-67]. The tubuloepithelial compartment of the kidney is the main source of local complement production during rejection, with infiltrating cells also contributing to the levels [65, 68]. For example, 10% of circulating C3 found during clinical episodes of rejection have been reported to originate from the kidney allograft compared to the 5% found in stable kidney allograft function [66]. Interestingly only 1% of circulating complement detected is of myeloid origin following a bone marrow transplant [69]. This supports the role of organ produced complement detected in mouse studies, and its potential of initiating or sustaining local injury through these mechanisms thereby introducing novel therapeutic targets.

Donor derived complement in murine kidney transplantation has been reported to be essential for acute allograft rejection [16]. Transplantation of kidneys from C3-deficient donors resulted in an increased rejection free survival supporting a role for donor-derived C3 in murine transplant rejection [16]. These models illustrate how complement damages the renal parenchyma directly and indirectly by enhancing the recipient's T cell response in a C3 dependent manner [31, 47]. A similar phenomenon is reported in cardiac transplants with activation of the classical and alternative pathways via inhibition of the complement regulator CD55 [70-72].

C3 downstream products C3a and C5a have been shown to be important in antigen presentation thus further stimulating T cell responses against alloantigens, inducing rejection [10, 14, 39, 41, 47, 53, 73, 74]. Recipient T cells are known to be primed within the first 24 hours following transplantation as donor APCs migrate to the recipient lymphoid system. Once primed, alloreactive T cells enter the graft resulting in graft rejection. Studies have reported the susceptibility of APCs to complement products generated through cleavage of C3 [10, 75]. In addition, APCs stimulated with C3a or C5a increased their ability to effectively stimulate alloreactive T cells via nuclear factor- κ B (NF- κ B) signalling [39, 41]. APCs such as macrophages and dendritic cells (DC) are known to produce C3 and C5 [76]. C3a/C3aR and C5a/CD88 upregulate and release IL-12 and IL-23 from macrophages and DC. Co-stimulating molecules CD80, CD86 are also increased [49, 74]. APCs deficient in C3aR/CD88 reduce circulating IL-12 levels and CD80, inducing weak T cell responses promoting graft survival. Murine studies have shown macrophages from CD55 deficient mice induce stronger T cell responses and IL-12 than wild types. Recently the effect of donor derived APCs C3a/C5a production was also seen to decrease human T cell alloresponses when small interfering RNA (siRNA) knockdown of DC expressed C3 in human cultures. Following knockdown of DC expressed CD55, T cell proliferation was observed as increased levels of C3a/C5a were detected supporting donor APCs as an important source of complement [77].

In addition to APC, T cells express a number of complement receptors such as C3aR and possibly CD88 that may be activated against major histocompatibility antigens directly by signals from soluble and membrane bound products of complement [12, 38]. CD88 signalling has also been reported to be beneficial in altering the balance of the death receptor CD95 (FAS) and the anti-apoptotic factor B cell lymphoma 2 (BCL-2) thus improving T cell survival by promoting T cell proliferation and inhibiting apoptosis [38]. Proinflammatory cytokines can stimulate endothelial cells to produce C5a with activation of the alternative pathway. This locally synthesised C5a induces proliferation and expansion of CD8+Tcells [53]. CD4+Tcells are able to interact with tubuloepithelial cells via interactions between C3b and CD35 leading to enhanced CD4+Tcell alloreactivity [78]. Complement activation has also shown CD4+Tcells help CD8+Tcell expansion during renal allograft rejection [72].

Tregs have long been reported to have a central role in the development and maintenance of allograft tolerance [79, 80]. Complement is known to regulate Tregs via signalling through C3aR and CD88 resulting in inhibition of Treg function [81]. In this murine study C3aR/CD88 antagonists enhanced induced Treg (iTreg) activity promoting allogenic skin graft survival. CD88 antagonist has also been reported to enhance human iTreg production and stability in murine recipients of human

peripheral blood mononuclear cells [82]. These complement receptors may therefore be novel targets for blocking complement signalling in order to facilitate iTreg mediated tolerance in allografts. Complement has thus been likened to a double-edged blade that enhances T-cell mediated immunity but yet is required for T cell tolerance induction, in different model systems. The inhibition of complement currently presents a challenge for predicting and inducing the desired effect on Tregs.

2.2.2 Antibody mediated rejection (AMR)

Both the production and effect of alloantibodies on tissue injury in transplantation are dependent on complement. This is increasingly important, as the development of HLA antibodies formed post transplantation have been correlated with poor allograft function. C3 has long been described as an opsonin that effectively maintains antigens for B cell stimulation via receptor CR2 for antibody production [83-85]. Complement deficient mice undergoing bone marrow transplantation have subsequently regained their ability to produce antibodies via donor C3 producing monocytes repopulating the central lymphoid system of these animals [86, 87]. It has been reported that complement deficient mice are unable to produce high affinity IgG responses against MHC antigens in skin grafts. They lack C4 dependent cleavage of C3, suggesting the classical or lectin pathway affects the anti-donor IgG response [88].

Donor specific antibodies (DSAs) are antibodies that in vitro have the ability to activate complement (i.e. bind C1q). DSAs that bind donor HLA molecules may be complement fixing resulting in complement activation via the classical pathway and C4 deposition on the graft with subsequent split produce C4d. In contrast, non-complement fixing DSAs have not been associated with renal transplant decline [89]. C4d has long been described as a feature of AMR, however C4d independent AMR has since been described and thus a recognised effort is also being made to prevent alloantibody production to prevent AMR [90]. B cell depletion using Rituximab (anti CD20 monoclonal antibody) has been reported to decrease AMR and improve graft survival [91]. A combined approach of B cell depletion and complement suppression could prove to be effective in promoting long term graft survival.

2.2.3 Chronic AMR (CAMR)

The development of new immunosuppressive therapies has decreased the incidence of cellular rejection and acute AMR making chronic AMR a predominant cause of late allograft loss [92, 93]. The presence of HLA-DSA is linked to lower levels of functioning grafts from deceased donors, with 70% functioning at 5 years compared with 80-90% graft survival in those with no antibodies. The typical histological feature of CAMR in the kidney is described as transplant glomerulopathy (TG), where its presence has been associated with preformed DSAs present at transplantation [94]. Currently it is thought CAMR arises from an initial injury involving DSAs and complement activation whose chemotactic effects result in inflammatory cell infiltration. It is postulated that TG is the consequence of continuous peritubular glomerulitis and capillaritis or that low levels of DSA and complement are present in the graft, but current assays are unable to measure them [95]. There are also reports of endothelial cell and smooth muscle cell activation by DSAs alone in in-vitro studies thereby suggesting complement-independent

mechanisms may contribute to chronic endothelial cell damage [96, 97]. The treatment and prevention of CAMR therefore continues to be a challenge.

2.3 Negative regulation of the immune system by complement - a role in tolerance?

Normally the C3b receptor known as CD46 modulates human CD4+T cells towards an immunosuppressive phenotype producing IL-10. In autoimmune diseases this mechanism is lacking and may explain how complement induced T regulatory type 1 cells (TR1 cells) may modulate T cell-mediated immunity against self-antigens [98]. People with complete complement C3 deficiency have low numbers of TR1 cells with impaired CD46 T cells. Complement activation has also been found to play a role in graft tolerance by inducing non-responsive T cells [99].

Induction of graft tolerance has been described in murine studies. Skin grafting performed on male to female mice initiates a T cell response to minor histocompatibility antigen H-Y resulting in rejection. Rejection was seen to be more aggressive in female C3 deficient mice. Induction of tolerance occurred following nasal immunization of the mice with an H-Y peptide whose reaction is also dependent on C3 thereby suggesting that complement activation is necessary to induce nonresponsive H-Y specific T cells [99]. C3 appears to be important in the development of inhibitory T cells that promote tolerance against alloantigens however this effect seems to be limited to minor histocompatibility mismatches between the donor and recipient. This tolerogenic effect has not been described in MHC-mismatched grafts and thus the role of C3 in tolerance currently remains unclear.

2.4 Complement and renal fibrosis

C3 synthesis by renal epithelial cells has been reported to mediate proteinuria induced tubular damage and activate the renin-angiotensin-aldosterone system with a possible role in epithelial to mesenchymal transition [100, 101]. C3 knockout kidney allografts transplanted to wild-type were protected from proteinuria, toxin induced tubular damage and loss of renal function despite circulating C3 levels [102]. Activation of C3 from intrinsic renal cells could therefore contribute to the fibrosis seen in allograft failure in chronic rejection with donor derived C3 potentially playing an important role in its development.

2.5 Complement and the coagulation system

Both of these pathways are serine protease cascades with interlinking molecules, for example, C5a and C5b-C9 increasing tissue factor production from endothelial cells (ECs) leading to stimulation of the coagulation system and release of thrombin from pro-thrombin [103]. The lectin pathway enzyme MASP-2, can also cleave pro-thrombin to thrombin and activate fibrinolysis [104]. Thrombin, Factors XIa, Xa, IXa and plasmin are all described to cleave C3 and C5 showing how these pathways interlink and may initiate complement activation independently of established pathways [105]. The binding of alloantibodies to the donor organ endothelium triggers complement and coagulation cascades in a pro-inflammatory and pro-coagulant response mediated by C5a and C5b-C9. The EC response is variable causing immediate thrombosis and infarction of the graft or a lesser injurious process, in the presence of alloantibodies and complement.

ECs express CD46 and CD55 that inhibit the cleavage of C3 and C5 by the converting enzymes of the classical and alternative pathways. They also express CD59 that blocks the insertion of C5b-C9 into the cell membrane. In cardiac and renal transplants accommodation has been reported to arise from increased expression of these inhibitors in the allograft [106, 107]. Accommodation is defined as an acquired resistance of an organ to immune mediated damage thereby maintaining good allograft function [108]. Cultured human ECs upregulate CD55 and CD59 when stimulated with pathogenic antibodies, C5b-C9, thrombin and other pro-inflammatory molecules such as TNF [109, 110]. Other studies suggest anti-apoptotic proteins such as BCL-2 and haem oxygenase 1 (HO1) upregulate CD55 thereby enhancing endothelial resistance and promoting accommodation of the graft [111]. ECs also modulate T cell function and expansion through local production of complement and CD88 signalling on T cells seen in heart and kidney transplantation models [112, 113]. In view of this, manipulation of complement regulators may prove an effective treatment strategy in the treatment of antibody-mediated rejection.

3. New therapeutic targets

Appreciation of the relative roles of the peripheral and central complement production play in the development of IR/DGF, cell mediated rejection, AMR, CAMR, tolerance or renal fibrosis allows new therapeutics to be developed accordingly. This will also depend on the particular complement components being targeted.

3.1 Target - Donor organ derived complement

A number of methods are being developed on the basis of animal studies that one day may lead to modification of a donor organ to protect it from inflammatory or immunological injury that is initiated upon contact with recipient blood. A summary of new therapeutics are described in Table 2.

3.1.1 Small interfering RNAs (siRNAs)

siRNAs are double stranded RNA that are manipulated to selectively silence the gene expression of the target such as C3 or CD88 thereby protecting against post-ischaemic acute kidney injury [114]. They may also be introduced directly to the donor organ in preservation fluid prior to transplantation previously described in a murine model [35, 115, 116]. This approach has also used antisense oligonucleotides [116] however, high concentrations of this agent are required to induce an effect.

3.1.2 Complement regulatory proteins

Complement regulatory proteins that remain in the donor organ following transplantation may act as potential therapeutic targets, however this is limited by an inability to upregulate these factors and directly deliver these agents to the target organ [117]. An analogue of human complement regulatory protein CD35 has been developed that destabilises the C3 and C5 convertases affecting all complement pathways. In this molecule, a basic peptide with the ability to bind acidic phospholipid headgroups is combined with a myristoyl fatty acid group (Mirococept, APT070) allowing the active fragment of CD35 to attach to the outer leaflet of the cell membrane lipid bilayer. It is maintained there through electrostatic interactions at the cell membrane with the peptide amino groups. This enables the complement regulator to function locally. Previous studies have shown Mirococept to localise to endothelial and epithelial cell surfaces once infused through the renal artery in rat

and human donor kidneys [118, 119]. This resulted in a decrease in IR injury and less chronic vascular injury in rat donor organs. A clinical trial is currently underway to determine its efficacy in the prevention of IR injury in transplanted kidneys (EMPIRIKAL) [120]. The modification used with this CD35 fragment, known as “cytotoxic” and has also been applied to other complement regulatory proteins such as CD59 and CD55.

3.2 Target – Systemic complement

Eculizumab is a C5-specific antibody licensed for use in patients with paroxysmal nocturnal haemoglobinuria and aHUS [121, 122]. This antibody inhibits the formation of C5a and C5b-C9 and therefore can prevent the pore formation on the transplant endothelium [123]. Eculizumab has been shown to be effective in the prevention of AMR, in the reversal of established rejection and in the recurrence of aHUS in the kidney allograft [123-125]. Factor H synthesised in the liver and CD46, a transmembrane protein, are the commonest mutations found in aHUS that result in intrarenal thrombosis and haemolysis as the complement pathways are largely unregulated post-transplant. Eculizumab has been revolutionary in these patients allowing successful transplantation in this cohort of patients [125-128]. Eculizumab is also being tested for the prevention of delayed graft function (NCT01403389).

Murine studies have used a combination of the complement receptor CR2 that detects membrane bound active and inactive C3b and C3d, fused to a complement regulator CRRY or Factor H thus inhibiting complement activation at the targeted site. The CR2-CRRY combination drug inhibits all the complement pathways, whereas CR2-Factor H targets the alternative pathway, however both reduce post-ischaemic myocardial damage [129]. CR2-CRRY inhibition has also been described to reduce IR injury in cardiac transplants in recipients from deceased and live donors [130]. Recombinant human C1q antagonists have been tested in acute AMR in baboons and provide alternative targets in humans to block the early activation of complement [131].

A C3 antagonist – Yunnan-cobra venom factor (Y-CVF) given to HLA-sensitised rhesus monkeys following a HLA incompatible kidney transplant, maintained normal renal function. They had low levels of DSA and variable low level C4d deposition on biopsy up to 100 days later. In contrast, the non-treatment group developed early AMR and lost their grafts. It is unclear whether this can be extrapolated to human complement in view of species difference however it appeared proximal complement blockade may induce accommodation with grafts being preserved despite high levels of antibody [132].

Pexelizumab (Pex), is a recombinant short acting single chain variable fragment derivative of Eculizumab. Pex was being studied in a clinical phase III trial in patients with myocardial infarction with primary percutaneous coronary intervention or coronary artery bypass [133]. No difference in 30-day mortality was observed between treated and untreated patients and thus no further clinical trials have been undertaken. In addition, a neutralising C5 antibody utilizing a different epitope to Eculizumab has also been developed known as ‘Mubodina’ however sepsis and the development of inflammatory disease are side effects that have limited clinical trials [134]. In contrast, Compstatin is a cyclic tridecapeptide C3 inhibitor function that continues to be promising in clinical trials as a treatment for age-related macular

degeneration [135]. It binds C3 reversibly whilst inhibiting the convertase mediated cleavage of C3 to C3a and C3b affecting the classical and alternative pathways.

Recently new anti-complement monoclonal antibodies have entered into clinical trials. Anti-MASP-2 is a human monoclonal antibody that selectively inhibits MASP-2 thereby blocking the lectin pathway. There are ongoing clinical trials in aHUS and if successful this selective blockade may be associated with a lower infectious risk as the classical pathway would remain unaffected [135]. Previous murine models have shown a reduction in IR injury of the myocardium and gastrointestinal tract with blockade of MASP-2 [51]. Anti-C1s monoclonal antibody has been reported to inhibit HLA-antibody induced complement activation [136]. Anti-C1s may be useful in modulating effects of DSA by inhibiting complement deposition and split product formation arising from HLA class I and II seen in vitro, human clinical trials are pending. Anti-CD88 monoclonal antibody has been reported to dramatically decrease joint inflammation in a murine model of arthritis following a single treatment. It is thought to induce its effects by reducing neutrophil infiltration and activation whilst modulating circulating T cells [137]. A combination of these agents may provide effective therapy in the near future however the challenge of when and how long to use them for leads to the question of whether any biomarkers exist that may aid this decision making.

4. Biomarkers of effective therapy

Donor C3 has previously been described to be associated with the risk of graft dysfunction [138]. A microarray analysis of complement in donors prior to transplantation showed high levels of C3 in organs with a poor allograft function at 3 years follow up [19]. Poor graft function has also been associated with tubuloepithelial complement deposition on pre-implantation biopsies [138]. C4d deposition identified on transplant biopsies has been synonymous with AMR [139]. In addition, serological tests have been developed to determine complement fixing HLA-specific antibodies that have been reported to have a higher sensitivity to underlying episodes rejection [138]. Detection of serum anti-HLA antibodies binding C1q in kidney transplantation have also been associated with poor graft function [89]. Currently there continues to be a paucity of good complement biomarkers to guide new potential therapies.

MBL levels may also be used as lectin pathway complement biomarkers with higher risks of infections reported in those with low MBL expression post liver transplant reflecting underlying inactivity of the lectin pathway [140]. In kidney transplants, high levels of MBL correlate with aggressive rejection and graft failure [55]. CD55 has been reported to be a marker of renal allograft survival when expressed in the peritubular capillaritis of AMR [141] that could be used as a biomarker whose presence may herald cessation of complement inhibitory treatment. It is increasingly important with the development of complement inhibitory therapies to identify biomarkers that may help guide the clinician to determine when treatment should be held or restarted.

4.1 Biomarker limitation

The difficulty in measuring and detecting complement activity within grafts is more challenging over time as it remains unclear whether donor derived complement is lifelong. Serial biopsies are not an acceptable risk and are also subject to sampling

errors thereby limiting their usefulness in the clinical arena. Imaging techniques may track complement activity using conjugated CR2 with superparamagnetic iron oxide (SPIO) nanoparticles [142]. Alternatively, CR2 radiolabelled with ^{99m}Tc can be injected and followed with single-photon emission computer tomography (SPECT) imaging allowing real-time visualisation of post-ischaemic myocardial injury that may further elucidate the function of major targets [143, 144].

5 Conclusion

In order to promote complement inhibition there needs to be a deeper understanding of the dual function of complement to promote and limit tissue damage whilst regulating T cell function that may help induce a tolerant state [99, 145]. Determining which triggers initiate the complement cascade in transplantation will provide new targets specific to the dominant complement pathway activated that may be inhibited to prevent allograft loss. The Lectin pathway is increasingly described in kidney transplantation and may allow new ligands on injured tissue causing this activation to be identified and subsequently targeted with new therapeutics. Complement inhibition continues to pose a theoretical risk of infection as a number of human complement regulators are receptors for human pathogens, thus a careful balance needs to be achieved between benefit versus harm. Complement genotyping before transplantation may also be a helpful adjunct to stratify risk. In future we may also see effective ways of treating the donor graft prior to transplantation resulting in longer and more effective graft survival.

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Figures

Figure 1. The complement cascades.

The classical pathway is triggered by the C1 complex (C1q, C1r, C1s) binding to antibody or C-reactive protein on the activating surface, leading to the formation of the classical pathway C3 convertase from C4 and C2 (C4bC2a) and to subsequent cleavage of C3. The lectin pathway is activated by collectins (e.g. Mannose binding lectin, MBL) or ficolins that detect carbohydrate residues, IgA or IgM on the activating surface. Complexes formed between the lectin molecule and MBL-associated serine proteases result in the formation of C3 convertase (C4bC2a), as per the classical pathway. In contrast the alternative pathway begins with direct binding of C3b to the activating surface after spontaneous hydrolysis of C3 or following the deposition of C3b by the classical or lectin pathway. In this case, C3b binds factor B followed by cleavage of factor B to form the alternative pathway C3 convertase (C3bBb). All three pathways form enzyme complexes (classical or alternative convertases) that cleave C3 into C3a and C3b and ultimately cleave C5 into C5a and C5b. C5b triggers the terminal pathway forming the membrane attack complex (C5b-C9), which creates a pore in the cells membrane resulting in cell lysis and death. Specific cell surface receptors detect C3a, C5a, iC3b, C3b and C3d. Complement regulators (CR) include: CD35 (CR1), CD46 (MCP), CD55 (DAF), C4BP (C4b-binding protein) and complement Factor H, which function by accelerating decay of the C3 and C5 convertases or by acting as cofactors for the proteolysis of these convertases by Factor I, or have both of these actions; and CD59 (Protectin), which binds C8 and inhibits membrane attack complex formation Table 1 provides further details of CR.

Tables

Table 1 Function of membrane bound and soluble complement regulators and whether they are specifically synthesised within the kidney [146]

Complement regulator	Function	Synthesised in the kidney
CD55 (Decay accelerating factor DAF) – membrane bound	Membrane bound complement regulator accelerates cell surface assembly of C3 convertase	Yes- podocytes/EC/ juxtaglomerular cells
CD46 (membrane cofactor protein MCP) – membrane bound	Cofactor with Factor I inactivates C3b and prevents the formation of C3 convertase	Yes- renal tissues
CD59 (Protectin) – membrane bound	Inhibits C9 and MAC formation	Yes- all major cell types
CD35 (CR1) – membrane bound	Inhibits C3 convertase limiting amplification of complement cascade and MAC formation. Decay and cofactor activities	Yes –variable expression
FH (Factor H) –soluble	Exhibits decay accelerating and cofactor activity of alternate pathway	Yes –tubule cells
C4BP (C4b binding protein) –soluble	Accelerates the decay of C3 and C5 convertase and cofactor activity of classical pathway	Podocytes
CFH related protein-1	Competes with Factor H function with controversial studies suggesting additional C5 convertase inhibitor activity	No
FI (Factor I)	Regulates degradation of C3b and C4b	Yes –on microarray for CFI
Properdin	Stabilises the alternative pathway convertases	Yes – renal tissues
Vitronectin (S-protein)	Inhibits C5b-7 and MAC formation affecting adhesion proteins, fibronectin attachments and coagulation pathway.	Unknown – accumulates in glomeruli
CR1g (VSIG4)	Inhibits C3 on macrophages affecting phagocytosis and alternative pathway activation	Unknown

Table 2 New therapeutic agents directly or indirectly targeting components of the complement system in the classical, alternative, and lectin pathways.

Agent	Target	Studies
Recombinant human C1q inhibitor	C1q	Animal AMR
Anti-C1s monoclonal antibody (True North)	C1s	Animal inhibits complement activation from HLA Antibodies potential AMR treatment.

		Human clinical trial commencing.
Anti-MASP-2 monoclonal antibody (Omeros)	MASP-2	Animal –reduction in myocardial and gastrointestinal IR. Human clinical trial
Mirococept (APT070) CD35 (CR1) inhibitor	CD35	Rodent and pig animal studies, human (Phase 1 and Phase 2 trial)
Compstatin C3 inhibitor	C3	Human clinical trial –Age-related macular degeneration
Small interfering RNAs (siRNAs)	C3 or CD88	Animal model IR injury (84,85)
Eculizumab (anti-C5 antibody)	C5	ABOi/HLAi transplants Delayed graft function
Anti-CD88 monoclonal antibody	CD88	Animal –reduction in arthritis. Human clinical trial
CR2-CRRY, CR2- Factor H	CR2	Animal post-ischaemic myocardial damage