Multifaceted role of the complement system in health and disease: a focus on properdin

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General introduction

The immunity of transplantation

Our kidneys play an important role in the removal of waste products by filtering our blood. Chronic kidney diseases (CKD) affect the renal function over time, eventually causing kidney failure also known as end-stage renal disease (ESRD) (1). Patients experiencing ESRD need renal replacement therapy (dialysis) or renal transplantation. In this thesis, we will discuss the principals of renal transplantation and the immunological processes which affect the outcome and function of the transplanted organ. We focus on the interaction of the innate and adaptive immune system, centralizing the local role of the complement system.

Transplantation is the golden standard for the treatment of patients with end-stage renal disease (ESRD) (2). In the transplantation process, various parameters are known to affect the outcome of the transplanted organ. This includes the quality of the donor organ, the type of donation - either from living, deceased brain death (DBD) or deceased cardiac death (DCD) donors - the preservation conditions and human leukocyte antigen (HLA)-matching (3–5). When the donor organ is transplanted, it is connected to the circulation of the recipient. Blood factors, including immune cells and components of the complement system, are able to recognize the foreign organ, which contributes to the damage of the transplanted organ, eventually affecting graft function and survival (4,5). The various processes, like activation of the complement system, to which the transplanted organ is exposed, are further explained below.

Ischemia and reperfusion injury

An inevitable consequence of organ transplantation is the development of ischemia and reperfusion injury (IRI). The ischemic phase takes place when the organ is detached from the blood flow, resulting in a lack of oxygen, also known as hypoxia. In this process, the cell reduces metabolism or switches from aerobic to anaerobic metabolism, causing ATP depletion and mitochondrial damage. During transplantation, the circulation is restored and the organ is reperfused, generating reactive oxygen species (ROS), resulting in oxidative stress. These processes contribute to apoptosis and necrosis, causing renal tubular cells and endothelial cell injury. Furthermore, pro-inflammatory gene expression is increased and release of so-called damage-associated molecular patterns (DAMPs) is also increased. These factors are responsible for the recruitment of immune cells, e.g. infiltration of neutrophils, dendritic cells and macrophages, further contributing to tubular and endothelial cell injury. IRI is a risk factor for delayed graft function (DGF), the onset of acute kidney injury (AKI) and the progression to acute and chronic rejection (3,6–10).

Transplant rejection

The (damaged) foreign organ is exposed to and recognized by the immune system of the recipient, which can result into rejection of the transplanted organ. Several categories

of rejection are known, of which hyper acute rejection can occur within minutes after transplantation (10). To prevent this, the complement-dependent cytotoxicity (CDC) crossmatch is applied to screen for compatibility between donor and recipient. In this test, the serum of the recipient is added to lymphocytes of the donor. If this test is positive, the recipient carries antibodies which are able to recognize HLA-antigens present on the donor organ and the transplantation procedure will not be continued (11). For highly immunized patients, which are currently accumulating on the waiting list, alternative strategies have been developed, including plasmapheresis to remove antibodies (10,12).

Acute rejection occurs within days to weeks after transplantation, late acute rejection after 3 months and chronic rejection months to years after transplantation (10). Acute rejection can be induced by T cell mediated rejection (TCMR) and antibody-mediated rejection (ABMR) and will be further addressed below. For the diagnosis of rejection, a renal biopsy is necessary.

Cellular rejection: T cell mediated rejection (TCMR)

In T cell mediated rejection (TCMR), antigen presenting cells (APCs) present antigens from the donor organ to the T cells of the recipient. This results in T cell activation and infiltration into the transplanted organ, eventually resulting in allograft damage (10).

Dendritic cells are the most professional APCs, which play an important role in bridging innate and adaptive immune responses (13,14). Immature dendritic cells are capable of antigen capture, processing and presentation via major histocompatibility complexes (MHC, named HLA in humans). Furthermore, immature dendritic cells express pattern recognition receptors (PRRs), to sense pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (15). These pro-inflammatory factors induce dendritic cell migration and maturation (Figure 1A) (16). Maturation is necessary to become potent activators of T cells, central components of the adaptive immune system. Dendritic cells are able to process and present foreign antigens to receptors on T cells: extracellular antigens are presented via MHC class II on dendritic cells to T helper cells ($CD4^+$ T cells) and intracellular antigens via MHC class I on dendritic cells to cytotoxic T cells ($CD8^+$ T cells; "signal 1"). Extracellular antigens can also be presented in MHC class I via cross-presentation (17,18). The costimulatory markers B7-1 (CD80) and B7-2 (CD86) are upregulated during dendritic cell maturation and are necessary for the interaction with CD28 expressed on the T cell ("signal 2"). Cytokines like IL-12 are produced by the dendritic cell and will further orchestrate the T cell, e.g. in Th_1 responses ("signal 3") (13–15,19) (Figure 1B).

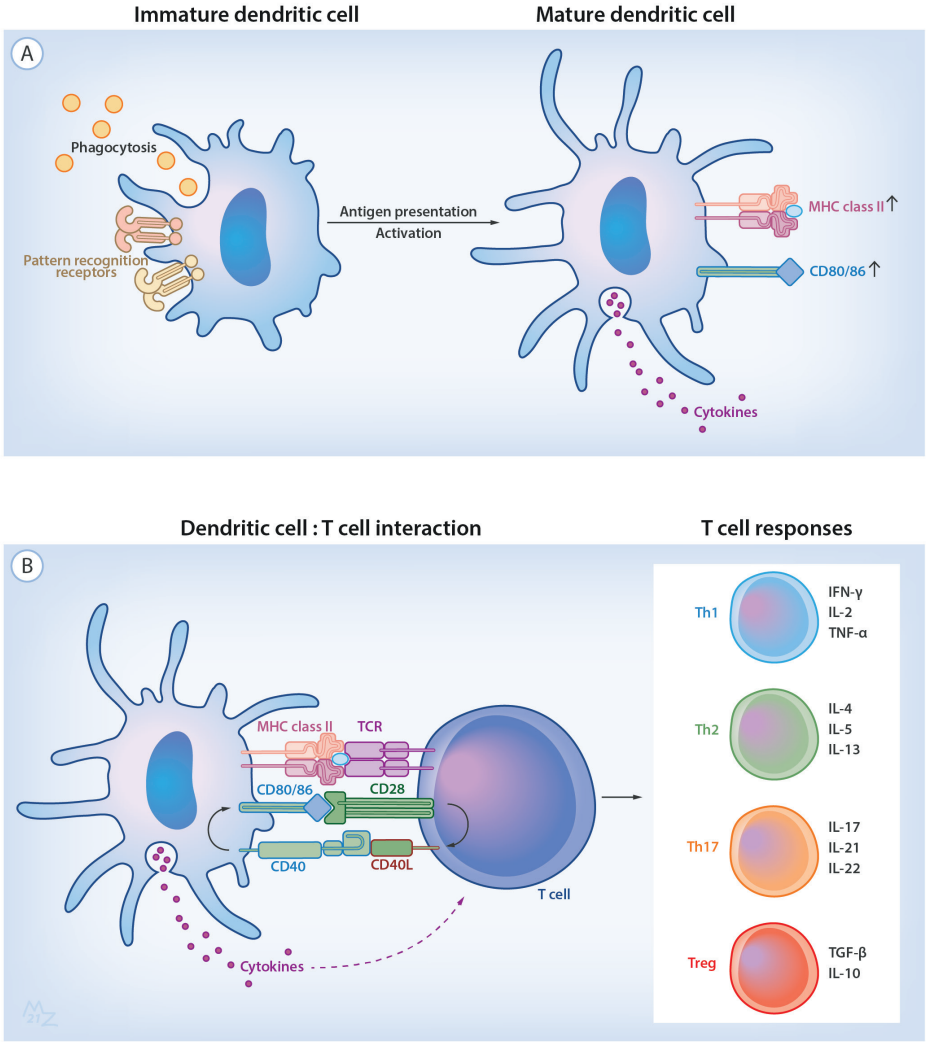

Figure 1: Schematic representation of dendritic cells - T cells interaction. (A) Immature dendritic cells express pattern recognition receptors providing the ability to sense the surrounding for foreign structures. Furthermore, immature dendritic cells are highly phagocytic, able to engulf antigens. These processes prime dendritic cells in their potential to present antigen by upregulation of MHC class II, and contribute to activation by the upregulation of CD80 (B7-1) and CD86 (B7-2) and cytokine production like IL-12. **(B)** Mature dendritic cells provide signals to activate naïve T cells. The T cell receptor (TCR) recognizes antigens presented in MHC class II by dendritic cells ("signal 1"), CD80/CD86 interact with CD28 on the T cell and via the interaction of CD40 and CD40L ("signal 2") and the dendritic cell produces cytokines like IL-12 ("signal 3"). The type of cytokines secreted by the dendritic cells play a determining role in the instruction of naïve T cells to a specified T cell lineage. Th1 cells secrete IFN- γ, IL-2 and TNF-α; Th2 secrete IL-4, IL-5 and IL-13; Th17 cells secrete IL-17, IL-21 and IL-22; and Treg TGF- β and IL-10.

In the process of transplantation, immature donor APCs, carrying the donor antigens, migrate towards the draining lymph nodes and spleen of the recipient. Matured donor APCs (e.g. induced by stress or injury of the organ) travel to the lymphoid organs and activate the T cells of the recipient. These T cells start to differentiate, proliferate and infiltrate the graft, causing damage to the transplanted organ (10). The process in which recipient T cells recognize intact MHC alloantigens presented by donor APCs is known as the direct pathway (20–22). In the indirect pathway, recipient APCs, which also circulate through the graft, present donor peptide fragments to recipient T cells (10,22–25). A third pathway involved in the recognition of donor alloantigens is the semi-direct pathway. In this pathway, intact donor MHC on the surface of recipient APCs, in which the MHC molecules are transferred between donor and recipient cells via a process called trogocytosis, are recognized by recipient T cells ((26–29) and reviewed by (22)). A schematic overview of these processes is shown in figure 2.

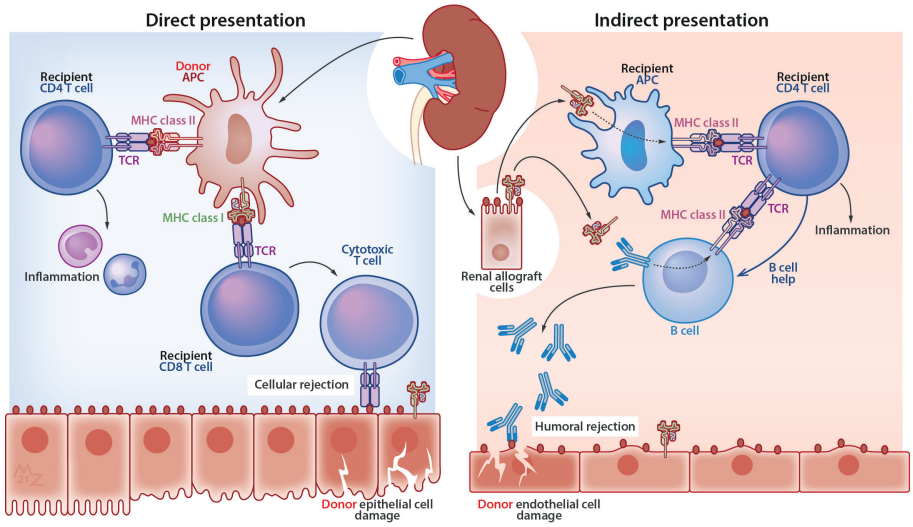

Figure 2: Schematic representation of the immune responses contributing to cellular and humoral rejection of a transplanted kidney. In the process of direct presentation, donor antigen presenting cells, like dendritic cells, resided in the donor organ. Upon transplantation, these cells present MHC alloantigens to $CD4^+$ T cells of the recipient, thereby activating the T cells, contributing to inflammation. In addition, donor APCs can present alloantigen via MHC class I to recipient $CD8^+$ T cells, generating cytotoxic T cells, contributing to epithelial cell damage and cellular rejection. In the process of indirect presentation, recipient APCs present donor peptide fragments to recipient $CD4^+$ T cells. B cells can also present donor antigen in MHC class II to recipient T cells, where these T cells provide help to B cells to become antibody secreting cells. These antibodies recognize antigens expressed in the kidney, for example on the endothelial cells, and will eventually result in humoral rejection of the organ.

Humoral rejection: antibody mediated rejection (ABMR)

Antibodies, either pre-existing before transplantation due to sensitization by exposure to alloantigens after blood transfusions, pregnancies, previous transplantations, or generated *de novo* after transplantation, can be involved in antibody-mediated rejection (ABMR). The antibodies are able to recognize antigens, for example HLA molecules and blood-group antigens (ABO) (Figure 2) (5, 10,30). Formation of the antigen-antibody complexes can induce activation of the complement system, which results in the deposition of C4d. C4d deposition on the peritubular capillaries (PTC) is routinely analysed in transplant recipients and is used as a diagnostic criterion for ABMR (30–33). In addition, immune cells like neutrophils, APCs and NK cells express Fcγ receptors which are able to interact with the HLA-antibodies bound to the endothelium, inducing immune cell activation which contributes to allograft rejection (34). Especially Fcγ receptors-mediated activation (CD16a/FcγRIIIa) of NK cells seems to be involved in ABMR (35).

To prevent the incidence of rejection, optimal MHC matching between donor and recipient is of importance. In addition, to prevent rejection, transplant recipients need lifelong treatment with immunosuppressive therapies. To prevent early acute rejection, patients can receive induction therapy targeting the IL-2 receptor (anti-CD25 antibodies, e.g. basiliximab and daclizumab) and/or a lymphocyte-depleting agent (e.g. rabbit anti-thymocyte globulin (rATG)) or alemtuzumab). As maintenance immunosuppressive therapy, a combination of various therapies can be used, often containing calcineurin inhibitors (e.g. cyclosporine and tacrolimus) preventing downstream transduction of signal 1 thereby inhibiting T cell activation, agents to prevent proliferation, for example mycophenolic acid (or mycophenolate mofetil) which inhibits purine synthesis, azathioprine which interferes with the DNA synthesis, mTOR inhibitors (e.g. sirolimus and everolimus) which binds rapamycin and inhibits T cell proliferation driven by IL-2, and corticosteroids (prednisone), all affecting and preventing cell proliferation (36,37). The innate immune system, including the complement system, also plays a role in TCRM and ABMR (4,38,39). The local role of the complement system will be further addressed below.

The complement system

A role for the complement system has been implicated in the context of transplantation (40). As described above, reperfusion of the transplanted organ leads to the exposure of the damaged cells and tissues to complement factors which are present in blood. This can result in activation of the complement system, aggravating the damage. Studies in mice showed that overexpression of so-called regulators of the complement system

can reduce damage after IRI, whereas mice deficient for these regulators were more prone to injury (41–43). In the context of APC-T cell interaction, it was shown that APCs like dendritic cells and macrophages are able to produce complement components, contributing to the local source of complement, which seems to be involved in T cell activation (44–48). Furthermore, as described above, activation of the complement system contributes to ABMR of the transplanted organ. Before the local role of complement is discussed, the complement system and its activation products will be introduced.

The complement system is an ancient system which plays an important role in the first line of defence against microorganisms and in the removal of dead cells. This system comprises three pathways, the classical (CP), lectin (LP) and alternative pathway (AP), consisting of over 30 proteins which are predominantly produced by the liver, and are activated in an enzymatic fashion (49,50). The pathways are initiated by various triggers. The CP is activated upon recognition of antibody-antigen complexes by C1q. The LP is activated by the binding of mannan binding lectin (MBL) or ficolins to sugar groups, exposed on the surfaces of pathogens. The AP can be activated spontaneously, via a process called tick-over (51–53). During non-enzymatic hydrolysis of the thioester of C3, C3(H_2O) is generated and binds factor B (FB) (54). The bound FB is cleaved by factor D (FD), generating a fluid-phase C3 convertase (C3(H_2O)Bb), which can cleave additional C3 molecules into C3b. C3b is able to interact with cell surfaces, binds FB which will be cleaved by FD, generating more C3 convertases (C3bBb), resulting in additional complement activation (49,50). Activation of the CP and LP pathway will also result in the formation of a C3 convertase, namely C4b2a.

Cleavage of C3 generates C3a, an anaphylatoxin involved in the recruitment of immune cells, and in C3b. Deposition of C3b on the cell surface functions as an opsonin by targeting foreign and altered host cells for removal by phagocytes. Upon a certain density of C3b deposition on the cell surface, the C3 convertase can interact with C3b for the generation of C5 convertases (C4b2aC3b for CP/LP and C3bBbC3b for the AP) (50). The C5 convertases can cleave C5 into C5a, an important anaphylatoxin for the recruitment of immune cells, contributing to inflammation. The formed C5b can interact with C6, C7, C8 and multiple C9 molecules, forming the membrane attack complex (MAC / C5b-9), a pore involved in the lysis of cells (Figure 3) (49,50).

Complement: a local role in bridging innate and adaptive immunity

The local role of complement in the regulation of T cell allo-immunity was shown for the first time in 2002. In a transplant model, allogeneic kidneys from C3 deficient mice were transplanted into a wild type (WT) recipient. Transplantation of C3 deficient kidneys

resulted in less rejection and a better renal function when compared to transplanted WT kidneys (55). In addition, it was shown that local donor derived C3 affected the outcome of the transplanted organ by regulating T cell responses, and was not affected by the systemic C3 levels present in the recipient (55).

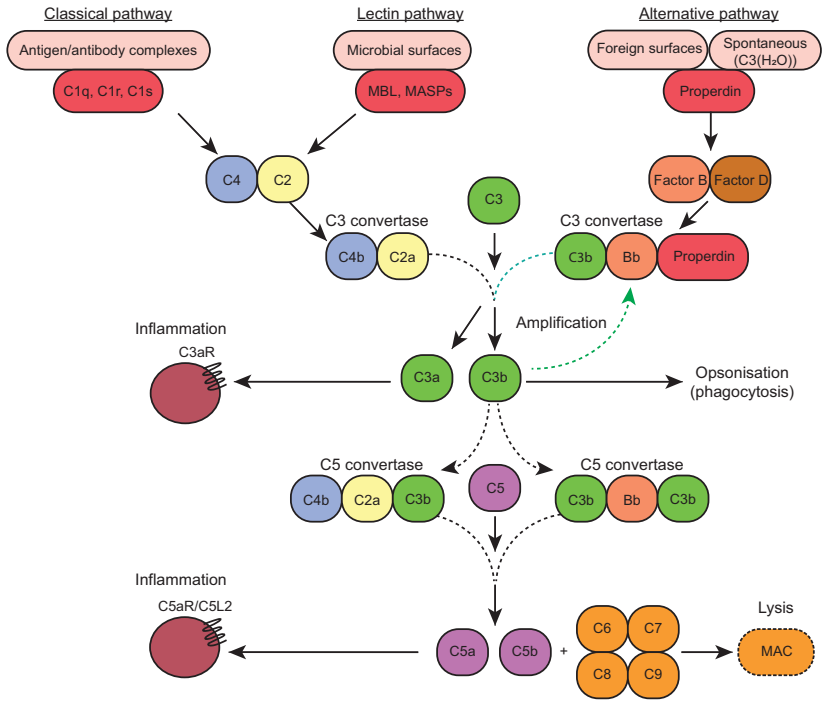

Figure 3: Schematic overview of the activation of the complement pathways. Activation of the CP, LP and AP results in the generation of C3 convertases, C4bC2a and C3bBb, respectively. C3bBb can be stabilized by properdin, increasing the half-life of the convertase. C3 convertases cleave C3 into C3a, an anaphylatoxin involved in the recruitment of immune cells by binding to its C3a receptor (C3aR). In addition, C3b is formed, which is involved in opsonisation and contributes to the amplification of complement activation via the AP by generating additional C3bBb convertases. Furthermore, C3b is involved in the generation of C5 convertases, C4b2aC3b for the CP/LP, C3bBbC3b for the AP. C5 convertases cleave C5 in C5a, an anaphylatoxin involved in the recruitment of immune cells interacting with the C5a receptor (C5aR). C5b is also generated, which interacts with C6, C7, C8, C9, generating a membrane attack complex (MAC) involved in lysis of cells.

APCs are able to produce, secrete and respond to complement factors (44), and a role for complement during the interaction of APCs and T cells has been implicated. C3 synthesis by dendritic cells is required for T cell priming and activation (56). C3a and C5a derived by dendritic cells contributed to T cell activation, since lowered C3a and C5a production, induced by siRNA knockdown, led to reduced T cell activation (46). Similar effects were observed by the blockage of the C3a receptor (C3aR) and C5a receptor

(C5aR) on dendritic cells (57,58). Furthermore, reduction of complement regulator DAF contributed to increased local complement activation and the production of C3a and C5a, resulted in an enhanced T cell activation and proliferation (46,59) (Figure 4A). These results indicate that local complement plays a role during APC-T cell interaction (60,61).

Next to C3aR and C5aR1 (and C5L2), APCs also express other complement receptors (CR). CR1 (CD35) is amongst others expressed by erythrocytes, monocytes, macrophages and B and T cells (62). CR1 binds C3b and C4b-opsonized cells. In addition, CR1 functions as a complement regulator by competing for C3b binding with FB and functions as a co-factor for FI. CR3 (CD11b/CD18) and CR4 (CD11c/CD18) are amongst others expressed by macrophages, monocytes, neutrophils and dendritic cells and bind to iC3b. The complement receptor of the immunoglobulin superfamily (CRIg), mainly expressed by tissue resident macrophages like liver Kupffer cells, is able to bind to C3b, iC3b and C3c (44,63–66). B cells express CR2 (CD21), and binding of C3d(g) and iC3b is a very important B cells stimulus (66). Recognition of C3b and iC3b via CRs on cells will result in additional immune responses, including phagocytosis and adaptive immune modulation (62,67–70) (Figure 4B).

Regulation of complement activation

The AP can be activated spontaneously via a process called tick-over (51–53). The formed C3b is able to interact with the cell surface of foreign and host cells. In the latter, the deposition and activation of the complement system is undesirable, such that tight regulation is necessary (64). Both membrane bound and soluble regulators are involved in the regulation of complement activation. Regulation can occur at the initiation phase, for example by C1-inhibitor (C1-INH), which inactivates the proteases involved in CP and LP activation (71). Further regulation of the CP and LP is provided by C4b-binding protein (C4BP), which functions as a cofactor for factor I (FI), involved in the inactivation of C4b, generating iC4b (72).

Decay accelerating factor (DAF; CD55), membrane cofactor protein (MCP; CD46) and membrane attack complex regulator (CD59; also known as protectin) are membrane bound regulators. DAF is able to bind to C3b and C4b as part of AP and CP/LP C3 convertases, thereby inducing the decay of these convertases (Figure 5, panel 1). MCP contains cofactor activity, helping FI in the cleavage of C3b and C4b into their inactive forms, generating iC3b and iC4b (64,65,73,74) (Figure 5, panel 1). Protectin (CD59) inhibits the interaction of C8α with C9, thereby preventing the formation of a functional C5b-9 pore (75,76) (Figure 5 panel 3). In addition, soluble regulators S-protein (also known as vitronectin) and clusterin are involved in the inhibition of the assembly and the insertion of the MAC complex, respectively (64,65) (Figure 5, panel 4).

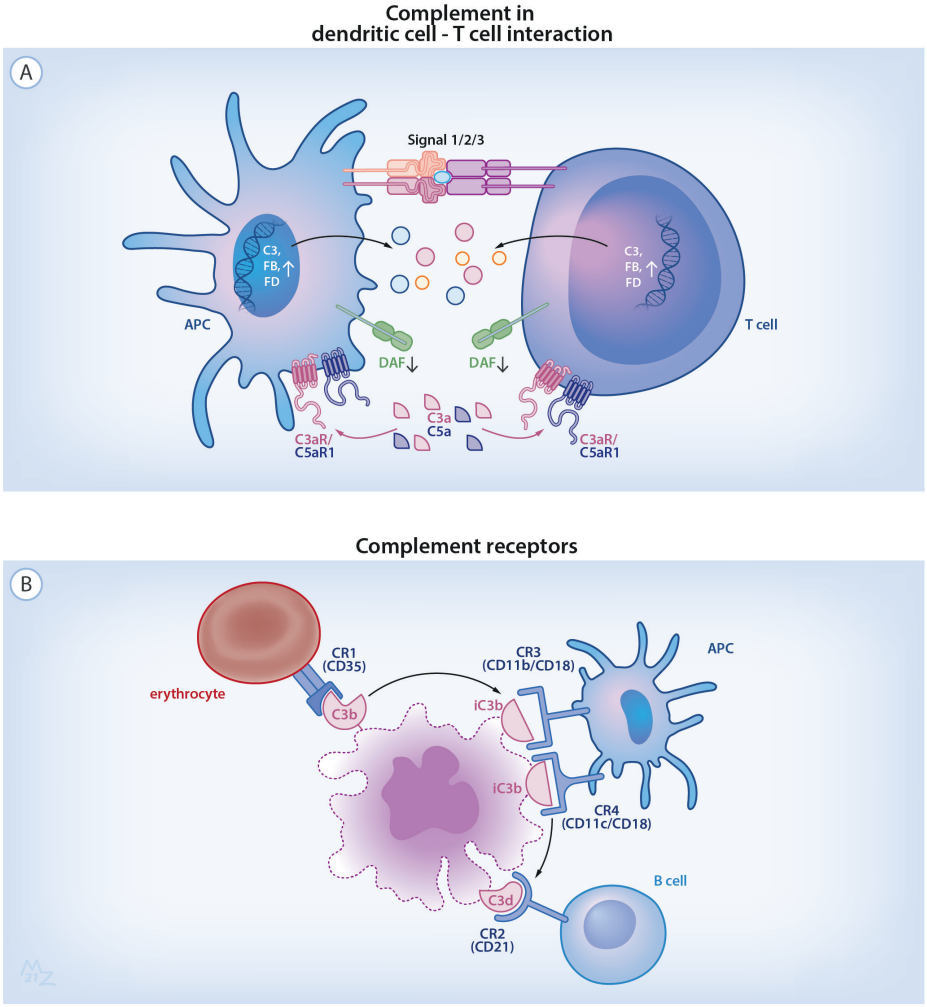

Figure 4: Schematic representation of the interaction of dendritic cells with T cells and the contribution of the complement system. (A) Local role for complement during the interaction of dendritic cells and T cells. Both cells are able to produce complement factors, which are upregulated during the interaction of the cells. Complement factors are released and membrane-bound regulator DAF is downregulated, allowing local complement activation resulting in the generation of the anaphylatoxins C3a and C5a. The anaphylatoxins are able to bind to C3aR and C5aR1, which are expressed on both types of cells, contributing to further activation of the cells. **(B)** Activation of the complement system results in the generation of C3b, which functions as an opsonin and can be deposited on foreign surfaces. C3b is recognized by erythrocytes via complement receptor (CR) 1 (CD35). Regulation, both by soluble and membrane bound regulators, will convert C3b into iC3b, which can be recognized by antigen presenting cells (APCs) via CR3 (CD11b/CD18) and CR4 (CD11c/CD18). Recognition of deposited C3b fragments is involved in inducing phagocytosis. Further degradation of iC3b results in C3d, which can be recognized by B cells via CR2 (CD21), and is important for the facilitation of antibody formation.

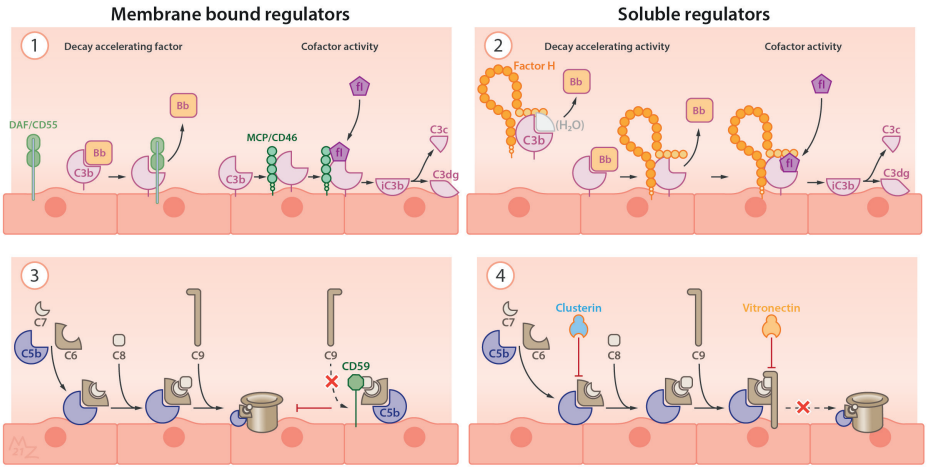

Figure 5: Schematic overview of complement regulatory proteins. (1) Membrane bound regulator DAF is responsible for the decay of the C3 convertases by the removal of Bb (and C2b), but keeps the deposited C3b/C4b accessible for the formation of new C3 convertases. MCP contains co-factor activity to help factor I in the cleavage of C3b into iC3b. Further degradation will result in the formation of membrane bound C3dg and soluble C3c. **(2)** Soluble regulator factor H contains decay accelerating activity for the removal of Bb from C3b to dissociate the C3 convertase. Factor H also contains co-factor activity, helping factor I in the degradation of C3b into iC3b and eventually C3dg and C3c. **(3)** The membrane attack complex is generated by the association of C5b, C6, C7, C8 with several molecules of C9. Pore-formation is prevented by membrane-bound regulator CD59, which prevents the interaction of C8α and C9. **(4)** Soluble regulator clusterin inhibits sC5b-7 and vitronectin inhibits sC5b-8, preventing the assembly and insertion of the membrane attack complex.

Soluble complement regulator: factor H

Factor H is a soluble complement regulator, involved in the regulation of AP activity. Like most other complement components, factor H is predominantly produced by the liver. Factor H consists of 20 complement control protein (CCP) domains, also known as short consensus repeats (SCRs). Specific SCRs are able to bind glycosaminoglycans (GAGs), which are expressed on host cells but absent on foreign cells, thereby preventing complement activation on host cells by exerting decay accelerating activity (DAF) and co-factor activity (77–82) (Figure 5, panel 2).

The factor H gene is located in the regulation of complement activation (RCA) cluster at chromosome 1, where also five complement factor H-related (FHRs) genes are located. These FHRs share some of the regions which are also found in factor H (83). The precise function of the FHRs remains to be elucidated. Some studies showed that these proteins contain complement regulatory functions, whereas others showed that the FHRs are able to compete with factor H for C3b binding, thereby affecting complement regulation (84,85).

Alternative splicing of the factor H gene results in the generation of factor H like-1 (FHL-1) (86–88). FHL-1 consists of SCR 1-7, similar to the domains present in factor H, however, at the C-terminus four amino acids (SFTL) are located. This means that FHL-1 contains complement regulatory activity in SCR 1-4, but lacks surface binding domains SCR 19-20. A schematic overview of factor H, FHL-1 and FHRs and their overlapping domains are shown in figure 6 (figure based and adapted from (89)). Mutations in or antibody formation against factor H are involved in the onset of various diseases. In contrast to the negative regulators of the complement system, also a positive regulator of the complement system is known, namely properdin, and will be further introduced and explained below.

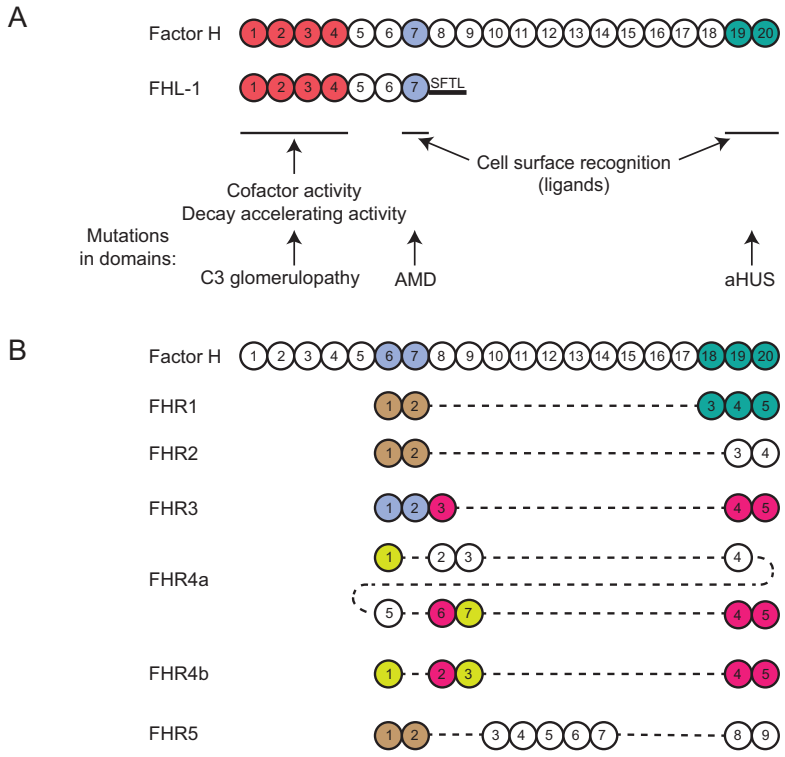

Figure 6: Schematic representation of the overlapping domains of factor H, FHL-1 and the factor H related (FHR) proteins. (A) Factor H comprises of 20 short consensus repeats (SCRs), containing both cofactor activity and decay accelerating activity (SCR1-4). In addition, domains for cell surface recognition are known. Alternative splicing of the factor H gene results in the generation of a factor H like protein (FHL)-1, of which the N-terminal SCR1-7 domains completely overlap with the ones of factor H, however, FHL-1 lacks the C-terminal domains. At the C-terminus, FHL-1 contains a short stretch of 4 amino acids which distinguishes FHL-1 from factor H. Mutations in various domains (indicated with the arrows) affect the complement regulatory functions of factor H and are associated with diseases like C3 glomerulopathy, age related macular degeneration (AMD) and atypical haemolytic uremic syndrome (aHUS). **(B)** Schematic overview of the overlapping domains of factor H with the factor H related proteins (FHRs). Overlapping colours indicate the similarity between the various SCR-domains. Figure based on and adapted from (89).

Properdin: the positive regulation of complement activation

Properdin was discovered in 1954 by Louis Pillemer (90) and is the only known positive regulator of the AP, stabilizing the C3 convertase. Due to stabilization, the half-life of the convertase is increased five to ten times (91). Individuals with a properdin deficiency are prone to meningococcal disease (92). Properdin deficiency has also been described to play a role in other diseases, for example in otitis media and pneumonia (Reviewed by (93)).

In contrast to other complement factors which are predominantly produced by the liver, properdin is mainly produced by myeloid cells. Neutrophils contain a pool of properdin in the secondary granules and dendritic cells are able to secrete properdin spontaneously (94,95). The serum concentration is ~5-20 µg/mL, which is relatively low when compared to the levels of C3. Properdin consists of 7 thrombospondin repeats (TSR), which can interact to generate dimeric, trimeric and tetrameric forms of properdin (96–99). A schematic representation of a dimer, trimer and tetramer form of properdin is shown in Figure 7.

Recently, crystal structures of properdin provided new insights into its role in C3 convertase stabilization (97,99). It was observed that two loops, referred to as stirrups, are formed, one from TSR5 and one from TSR6, which are able to fold around the C-terminus of C3/C3b. These stirrups might be responsible for the bridging of C3b and Bb interactions, thereby stabilizing the C3 convertase (99). Furthermore, domain TSR6 is able to sterically block the binding of factor I, preventing degradation (97,99).

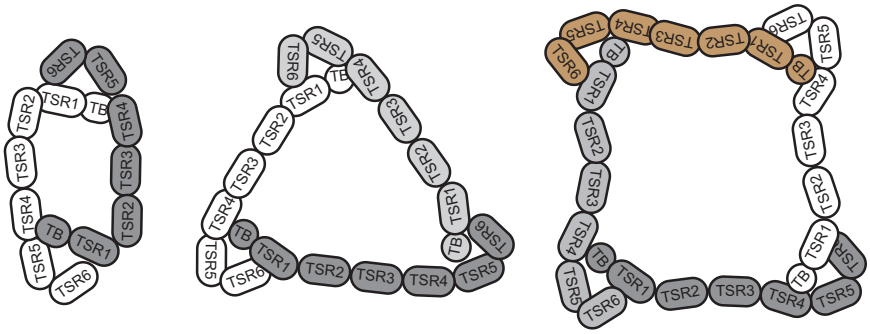

Figure 7: Schematic representation of a dimer, trimer and tetramer form of properdin. Interaction of properdin monomers will result in the formation of dimers, trimers and tetramers. TSR4 and TSR6 from one protomer are able to interact with the N-terminal domain of another protomer of properdin. Schematic representation based on (97–99).

There is some evidence that properdin could bind targets directly, contributing to properdin-initiated complement activation (Reviewed by (100)). Properdin is able to interact with the surface of bacteria (101) and early (102) and late apoptotic cells (103). In addition, properdin was found deposited in the kidney, binding renal tubular epithelial cells (104–106). Properdin also interacts with myeloperoxidase (MPO), a constituent of neutrophils (107), potentially contributing to local complement activation on neutrophil extracellular traps (NETs) (108,109). In contrast, others showed that upon freeze-thaw cycles, non-physiological forms of properdin can be generated. Non-physiological forms were able to bind to all sorts of cell surfaces, whereas the fractionated natural forms of properdin strictly bound to zymosan and necrotic nucleated cells, indicating that this should be taken into account when investigating properdin binding *in vitro* (110,111). Others showed that binding of properdin was dependent on initial C3b deposition (112,113). However, recently properdin was found deposited in the glomeruli of the kidney of a C3 deficient mouse, indicating that properdin was able to interact with surfaces in the absence of initial C3b deposition (114). The dispute of the role of properdin in the initiation of complement activation will be further addressed and discussed in this thesis.

Complement therapeutics

Dysregulation and over-activation of the complement system has been described as a contributor to the onset and/or progression of renal diseases. In addition, complement activation products were shown to mediate glomerulonephritis. Mutations in complement regulators cause problems in the proper regulation of complement activation (33). Inefficient regulation can result in overactivation of the complement system and can contribute to damage in multiple organs, of which the kidney is predominantly affected. In the context of atypical hemolytic uremic syndrome (aHUS), around 60% of the patients have mutations in complement genes. Most often the factor H gene is affected, causing problems in regulation of complement activation (33,115). In addition, autoantibodies against factor H are found in ~10% of the aHUS patients (33,116). Due to these events, regulation of complement activation is impaired, contributing to complement-mediated damage of the endothelium and eventually results in renal injury (117).

C3 glomerulopathy (C3G), comprising of C3 glomerulonephritis (C3GN) and dense deposit disease (DDD), is characterized by the dysregulation of the AP. C3G can progress to end-stage renal disease (ESRD) and various percentages for the progression into ESRD in affected children and adults have been reported (118–121). ~25% of C3G patients have mutations in complement genes, e.g. in regulators like factor H and FI, and

in activators like C3 and factor B (118). In addition, the formation of autoantibodies can contribute to disease onset or severity (118,120). ~80% of the DDD patients and ~50% of the C3GN patients have a circulating autoantibody, namely a C3 nephritic factor (C3NeF) (33,118). C3NeFs are able to stabilize the C3 convertase, increasing its half-life (122). C5 nephritic factors, targeting and stabilizing the C5 convertase, are also described (123). In some patients, factor H or factor B autoantibodies are found, however, these are less frequently present (118). Gain of function mutations in for example C3 and factor B can result in overactivation of the complement system, contributing to disease onset and progression. In patients who received a renal transplant, a high disease recurrence rate has been observed (118,124).

Due to the impact of dysfunction of the complement system in several diseases, complement inhibitors obtained great interest. Eculizumab is a monoclonal antibody that binds C5, thereby preventing its cleavage into C5a and C5b, inhibiting recruitment of immune cells and downstream effector functions by preventing the formation of the membrane attack complex (reviewed by (125)). Eculizumab and ravulizumab, a long-acting version of eculizumab, are approved for the treatment of paroxysmal nocturnal haemoglobinuria (PNH), an haemolytic disorder (126,127). In PNH, complement regulators DAF and CD59 are absent on erythrocytes, due to a mutation in the enzyme responsible for the synthesis of GPI-anchors, needed for DAF and CD59 linkage to the cell surface. Complement is activated on these erythrocytes, causing lysis, which results in anaemia (126,127).

Eculizumab is also approved for the treatment of aHUS (in 2011), myasthenia gravis (in 2017) and neuromyelitis optica spectrum disorder (in 2019) ((128) and reviewed by (126)). In 2021, pegcetacoplan, a pegylated compstatin analogue inhibiting C3, is approved for the treatment of PNH, thereby competing with eculizumab (129,130). Due to the complexity of the complement system, proteins, peptides and antibodies are being developed to targeting the complement system at various levels, e.g. at C3 or C5 as discussed above, factor B (LPN023, small molecule in C3G) or factor D (ACH-4471/ ACH-0144471, small molecule in C3G) or prevent C5a-receptor signalling by blocking the C5aR1 (Avacopan, small molecule, anti-neutrophil cytoplasmic antibody-associated vasculitis (ANCA-AAV)) (126). Complement inhibition is investigated for various diseases, including sepsis, systemic inflammatory response syndrome (SIRS), periodontal disease, ANCA-AAV, C3G, age-related macular degeneration and cancer (125,126). In addition, clinical studies investigate the application of complement inhibitors in transplantation, targeting complement activation at several stages. In a phase I/II randomized controlled trial, highly sensitized desensitized patients treated with C1-INH (Berinert®) did not develop AMBR ((131), NCT01134510). In addition, treatment with C1-INH reduced IRI

induced DGF and led to a study investigating safety and efficacy of C1-INH to prevent IRI/DGF in patients transplanted with a DCD-kidney. C1-INH treatment, intraoperatively and after 24 hours, reduced the need for dialysis (2-4 weeks post-transplantation) and improved long-term allograft function ((132), (NCT02134314). Post hoc analysis showed a lower incidence of graft failure in patients who are at risk for IRI and DGF when treated with C1-INH ((133) NCT02134314). CINRYZE, also a C1-INH, will be evaluated for the use as a donor pre-treatment strategy for the decrease of systemic inflammation and DGF incidence in expanded criteria donors (NCT02435732, not yet recruiting).

In the EMPIRIKAL study, efficacy of Mirococept (APT070), a recombinant complement inhibitor derived from human complement receptor type 1, was tested to examine its superiority compared to standard cold storage perfusion fluid in the prevention of DGF after transplantation of DCD allografts (ISRCTN49958194) (134).

Treatment of DCD transplant recipients with eculizumab showed that eculizumab was well tolerated, but the DGF rate was not different between control and eculizumab treated patients ((135) NCT01403389, NCT01919346, both terminated). Treatment of sensitized patients with eculizumab before reperfusion and on several days post-transplantation did not raise safety concerns and eculizumab could provide prophylaxis against acute AMR induced injury ((136), NCT01567085). In pediatric patients, treatment with a single dose of eculizumab pre-transplantation resulted in a better early graft function and improved graft morphology, however, a high number of early graft loss was observed ((137), NCT01756508). The effectiveness of eculizumab in combination with standard of care was evaluated for the treatment of patients who developed de novo DSA and have deteriorating graft function (NCT01327573, recruitment completed). Another study investigating the efficacy and safety of eculizumab for the treatment of subclinical ABMR in sensitized renal transplant recipients is withdrawn (NCT02113891).

Inhibition of properdin could prevent enhanced activation of the AP. In *in vivo* studies, it was shown that inhibition of properdin was protective in inflammatory arthritis, renal IRI, aHUS and PNH (138–141). Surprisingly, targeting of properdin in C3G mouse models was not protective (142,143). A monoclonal antibody targeting properdin (CLG561, Novartis) is tested as monotherapy or in combination with a C5 inhibitor (Tesidolumab/LFG316, Novartis) in patients with geographic atrophy/AMD (NCT02515942). Novelmed Therapeutics developed a small-molecule properdin antagonist for rheumatoid arthritis and a monoclonal antibody against properdin (NM9401) for the use in PNH (144).

Outline of this thesis

In this thesis we focused on the local role of complement in the context of renal transplantation, with a main focus on properdin. Since the local role for properdin remains to be established, we reviewed in **chapter 2** the presence of properdin in urine, serum and renal biopsies in patients diagnosed with various complement-mediated renal diseases.

In **chapter 3** we investigated whether complement factor properdin and complement activation products C3d, C4d and C5b-9 are deposited in renal biopsies from renal transplant patients and if there was a correlation with delayed graft function.

In **chapter 4** we focused on the local role of factor H and properdin by investigating the synthesis and production of properdin and factor H by human macrophages. The presence of FHL-1, the splice variant of factor H, was also examined.

In **chapter 5** we described the potential of properdin to interact with the surface of human monocyte derived macrophages and analyse the mechanisms of interacting with necrotic cells, thereby contributing to local complement activation upon exposure to complement components. In addition, the mechanism of cell surface interaction was examined, using several mimics of glycosaminoglycan structures which are expressed at the cell surface.

For the investigation of the local role of properdin during APC:T cell interactions, we developed an *in vitro* transfection system using short-interfering ribonucleic acid (siRNA) for the transfection of human monocyte-derived dendritic cells. In **chapter 6** we described that the type of medium used during the siRNA transfection of monocyte derived dendritic cells affected the maturation status of these cells, altering the allogeneic T cell responses. In **chapter 7** we described the local role of properdin in the context of APC and T cell interaction. Properdin levels were reduced in monocyte derived dendritic cells by siRNA, followed by co-culture with allogeneic T cells to determine the effect on T cell proliferation and activation. In **chapter 8** the presented results are discussed and summarized.

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