The complement system in kidney diseases

current therapeutic options and upcoming drugs

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Contents

Abstract

A part of the innate immune system is the complement system. The complement system consists of three pathways which can be activated in different ways. It turned out that the complement system is of great importance in kidney diseases, and especially the alternative pathway plays a major role. Currently there are two approved drugs on the market which can intervene with the complement system and show beneficial effects in certain diseases. These are the drugs eculizumab and C1 inhibitors. However, eculizumab is very expensive and like C1 inhibitors, not that effective in some diseases. Therefore, it is important to look at inhibition of the complement system in different levels, also to minimalize the side effects. Some studies have been done for other drugs on other levels then eculizumab and C1 inhibitors. One of these drugs is compastin, which interferes with the complement system at C3, which could possible cause many side effects. Another target which is studied now is properdin, which is a specific factor for the alternative pathway. This seems to be a promising target, because other pathways will stay intact and inhibition in vivo already showed some promising effects. Then, there are receptor antagonists. Discussed in this review is the C5a receptor antagonist CCX168, which will be tested in a clinical trial this year. And as last, polysaccharide glycosaminoglycans are mentioned as a future target, because they interfere a lot with the complement system and have shown some inhibiting effects. Overall, there are some promising studies for new drugs to intervene with the complement system. Besides that, new studies should mainly focus on specific inhibiting the complement system to prevent side effects as many as possible.

Introduction

To protect the human body against diseases, the body has several mechanisms which are together called the immune system. The immune system can be divided into an innate part and an adaptive part. The innate part as well as the adaptive part has a humoral section and a cell-mediated section. The innate immune system is the immune system every person has the same and is present from the moment a baby is born, stays the same during lifetime, and is relatively aspecific. The adaptive immune system is also present from the beginning, but this system will develop over time and is antigen-specific. Both systems can work side by side, but they can also work together or activate each other (1).

One part of the innate immune system is the complement system, which is a part of the humoral immunity. This system consist of large series of proteins, mainly produced in the liver, which in a cascade can activate each other. Complement can be activated in different ways, which leads to the activation of different pathways. The three pathways of the system are the classical pathway, the alternative pathway and the lectin pathway (1). These pathways will be discussed in detail in chapter 1. What all three pathways have in comment is the splicing of complement factor C3 along the pathway, which will lead eventually to cleavage of complement factor C5. The cleavage of these factors lead to opsonisation of the invading microbes by attaching to their surface and it will lead to an influx of other immune cells, because the part which will not bind to the surface of a cell or microbe, works as an anaphylatoxin (2). This already describes two functions of the complement system, opsonisation and attracting other immune cells. However, besides promoting cell lyses, the complement system is also capable of directly killing microbes itself (1).

It has become clear that the complement system plays a role in all kind of diseases, including renal disorders. For example, it seems to influence Alzheimer's disease, it contributes to autoimmune diseases like lupus erythematosus or rheumatoid arthritis and it also plays a role in kidney diseases, like C3 glomerulopathy and haemolytic uremic syndrome (HUS) (3, 4). Therefore, it is important to find drugs that can influence the complement system. A drug that already has been found and has been approved by the Food and Drug administration (FDA), is called eculizumab (3). Eculizumab already showed a very positive effect in the clinic, but there might be complement inhibitors that can be more efficient in specific cases (2, 4).
This review will focus on the question: what is the role of the complement system in kidney diseases. Further, it will also discuss today's therapies for controlling the complement system and it will briefly look at upcoming therapeutic interventions.

Chapter 1: the complement system

§ 1.1 The alternative pathway

As mentioned in the introduction, one of the three pathways of the complement system is the alternative pathway. By the activation of this pathway, the complement factor C3 plays an important role. In the other pathways, which will be discussed later, C3 will be cleaved, as a result of a cascade of reactions, in C3b and C3a. However, C3 is also, in a low level, constitutively activated in solution to generate C3b in a process called C3 tickover (1). When C3 is cleaved, the reactive thioester bond, which is present in the C3, flips out. This bond can covalently bind the surface of microbes or cells. When the C3b binds to a mammalian cell, it is directly degraded by regulatory proteins (will be discussed in paragraph 5). If C3b does not bind to a surface at all, it is inactivated by hydrolization of the reactive thioester bond, and complement activation cannot proceed (1).

The alternative pathway is activated when a C3b generated by the C3 tickover binds to a surface of a microbe. After this covalent binding, a plasma protein, called factor B can bind to the C3b, which forms the complex C3bB. Binding of factor B is followed by splicing of this factor by factor D, which results in C3Bb and a little fragment called Ba, which is released from the convertase complex. The complex which remains, C3bBb, is stabilized by properdin, a serum protein (5). This complex, C3bBb is called the alternative pathway C3 convertase. This convertase can cleave more C3 to produce more C3b. The C3b can bind, besides to a cell surface, to the C3bBb complex, forming the so called alternative pathway C5 convertase (figure 1). What happens after the production of the C5 convertase will be discussed in paragraph 4 of this chapter, because it will be similar for all three pathways (1).

Besides that the alternative pathway is activated by C3 tickover, it can also be activated by the other pathways. All the pathways of the complement system have the cleavage of C3 along the pathway and thus produce C3b. Factor B can bind this C3b, which leads to continuing of the alternative pathway (5). Therefore it can be said that the alternative pathway C3 convertase functions to amplify complement activation (1, 2).

These were options to activate the alternative pathway by C3, however recently has been found that the pathway also can be activated by properdin, a regulator of the alternative pathway (will be discussed in paragraph 1.5). Properdin can bind to heparin sulfate (HS) (the most abundant form of polysaccharides in renal tissue) which are present on almost all mammalian cell types and which also can be present on microbial cells (6). After binding to HS, C3b can bind to properdin followed by binding of factor B which results in continuation of the alternative pathway (7).

§ 1.2 The classical pathway

The classical pathway is initiated by antibodies (IgG or IgM) that have antigen bound (2). Important in this pathway is the complement factor C1, which is composed of the subunits C1q, C1r and C1s. The C1q subunit binds to the antibody, which activates the pathway. For the activation of the pathway by IgG, several IgG molecules are necessary, because each IgG molecule only has one Fc region and the C1q subunits has to bind two Fc regions to be activated (1). In contrast, IgM can bind two C1q molecules in one, because of its pentrameric structure. IgM can only bind C1q when it is also bind to an antigen, because the antigen causes a conformational change of IgM, which makes it possible to bind to C1q (1). The other subunits of the complement factor C1, C1r and C1s are serine proteases. Two molecules of each of these proteases together form a tetramer, which is wrapped around the radial arms of the C1q complex (1).

When the C1q complex binds to the Fc regions of IgG or IgM, it leads to activation of C1r, which cleaves and activates C1s (1). Next, complement factor C4 can bind to the Ig-associated C1q. C4 is then cleaved by the $C1r_2S_2$ complex, which results in two fragments, C4b and C4a. C4b forms a covalent bond (with a thioester bond, like C3) with the antigen-antibody complex or with the antigenic surface and C4a is released.

The next protein in the cascade is C2. This will bind to the C4b and is then cleaved by C1s which results in a C2a fragment, which stays attached to C4b, and a C2b fragment which is released. The C2a fragment and the C4b fragment together form the classical pathway C3 convertase (C4bC2a complex) (1, 3). This convertase can bind C3, which will be mediated by the C4b component and will then be cleaved by the C2a component (figure 1) (1).

Like in the alternative pathway, the generated C3b cleaved by the classical pathway C3 convertase, can bind to the convertase, forming the C4b2a3b complex (2). This complex, also known as the classical pathway C5 convertase can cleave C5, which will be further discussed in paragraph 1.4. Besides that the C3b produced by the classical pathway C3 convertase can bind to the convertase itself, it also can bind to cell surfaces or the antibody which initiated the complement activation, leading to more activation of the complement system (1).

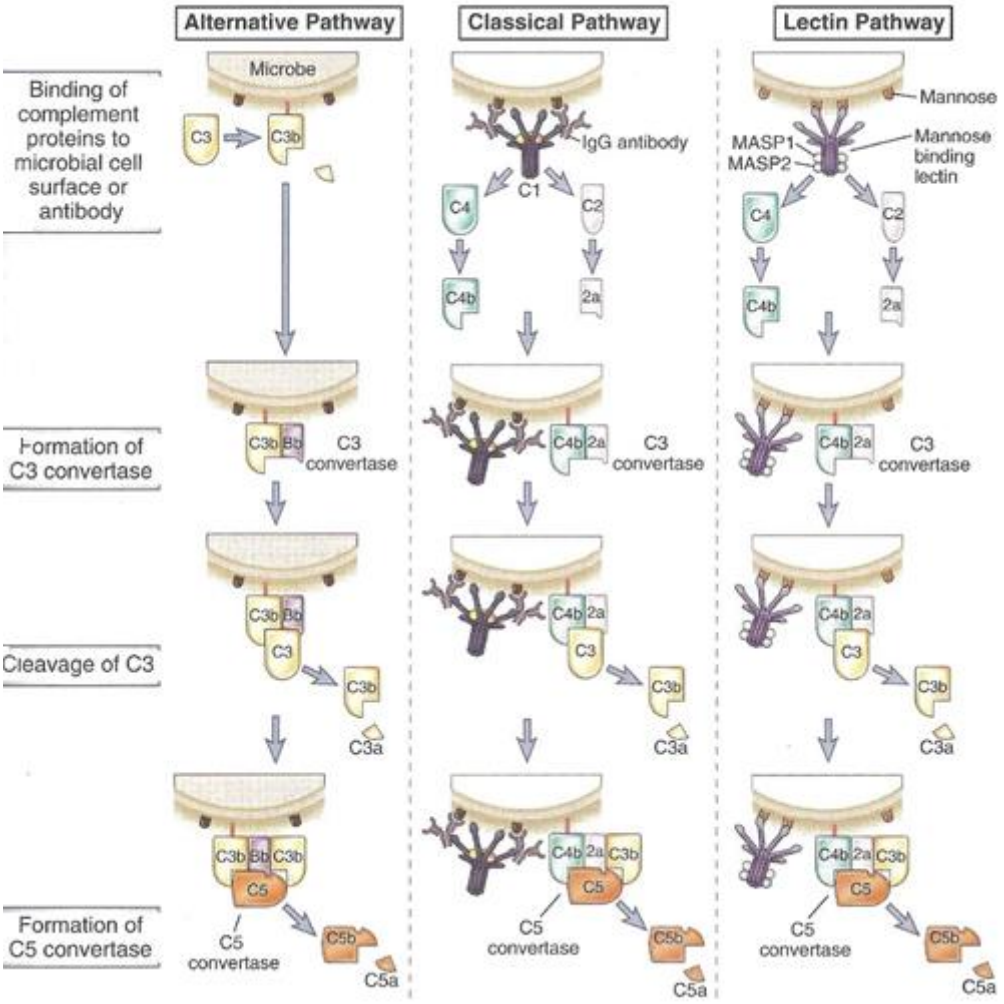

Figure 1: the pathways of the complement system. In this figure, the three different pathways and their complement factors are shown till the formation of the C5 convertase (1).

§ 1.3 The lectin pathway

In contrast to the classical pathway, the lectin pathway is activated in the absence of antibody. Lectins, like plasma mannose binding lectin (MBL), bind mannose residues on microbial polysaccharides and this activates the lectin pathway. However, microbial polysaccharides can also be bound by ficolins (2). As well as MBL, ficolins have an N-terminal collagen-like domain and besides this domain, ficolins also have a C-terminal fibrinogen like domain, which binds N-acetylglucosamine-containing glycans. MBL does not have this domain, but only have an N-terminal collagen like domain (1).

Like MBL structurally resembles C1q, this pathway has serine proteases which are structurally homologous to the C1r and C1s and they have similar functions. This are the MBL-associated serine proteases (MASPs). MASP1 and MASP2 can form a tetrameric complex, which can cleave C4 and C2. After cleavage of C4 and C2, the lectin pathway has the same continuation as the classical pathway (figure 1). Thus, the only difference between the lectin pathway and the classical pathway is the trigger and the serine proteases that cleaves C4 and C2 (1).

§ 1.4 After assembling of C5 convertase

So far, all three pathways of the complement system are described till the formation of the C5 convertase. After the formation of the C5 convertase, all pathways are following the same cascade, which eventually will lead to the formation of the membrane attack complex (MAC).The first step in the common pathway is the cleavage of C5 in C5a and C5b by the C5 convertase. The C5a fragment is released and works as an anaphylatoxin (8) and the C5b fragment remains bound to the complement factors on the cell surface (1). A temporarily conformation change of C5b makes it possible for complement factors C6 and C7 to bind, creating the C5b,6,7 complex, which is inserted into the lipid bilayer of the plasma membrane. By binding of C7 to C5b, it becomes a high-affinity receptor for C8, which is the next complement factor which binding to the C5b,6,7 complex, forming the C5b,6,7,8 (C5b-8) complex. To complete the formation of the MAC, the last complement factor to bind is C9, which is shown in figure 2. C9 is a serum protein that polymerizes at the site of the bound C5b-8 to form pores in the plasma membrane (1). The pores in the cell membrane make it possible for water and ions to move freely in and out of the cell, which leads to an osmotic rupture of the cells (1).

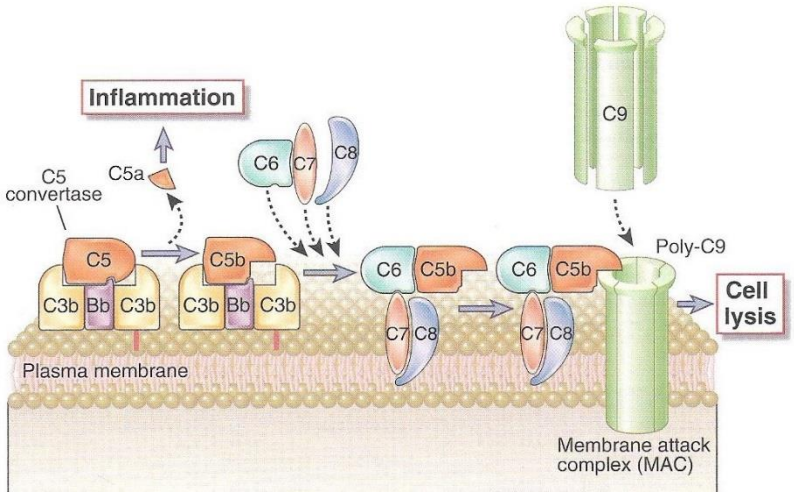

Figure 2: the formation of the MAC complex. After the C5 convertase is made, C5 is cleaved in C5a and C5b. C6, C7 and C8 bind to C5b, which leads to the binding of C9 which together forms the MAC complex. C5a works as an anaphylatoxin and induces inflammation (1).

§1.5 Regulation factors of the complement system

To prevent damage of the complement system to normal tissues and cells, there are proteins that regulate the activity of the complement system, these regulators can work on cell level but also in the circulation. Some of them will be discussed here. First, there is the C1 inhibitor (C1 INH), which can prevent the activation of the C1 complex in the classical pathway, by inhibiting the proteolytic activity of C1r and C1s. It inhibits the activity by binding to the tetramer of C1r and C1s, which results in a release of the complex from the C1q subunit and therefore the activation is ended. This is an inhibitor which works on circulation level. Other regulators of the complement system are factor H, C4-binding protein (C4BP), membrane cofactor for protein (MCP) and decay-accelerating factor (DAC). Factor H is a fluid phase regulator, as well as a surface bound regulator, it can bind to the negative oligosaccharide structures on cell surfaces to regulate the alternative pathway (9). C4BP, like C1 INH, is a plasma protein and can regulate the complement system in the circulating. MCP and DAC on the other hand, are regulators which are presented on cell surfaces (2). All these proteins are capable of binding to C3b or C4b, which prevents binding of other proteins to these complement factors. Therefore, the cascade cannot proceed. However, factor H only inhibits the binding of Bb to C3b and is thus only a regulator of the alternative pathway, but seen the fact that the alternative pathway has an amplifying function for the other pathways, factor H also has its indirect effects on the other pathways (1).

Another way of controlling the complement system is by cleaving C3b by circulating factor I in combination with cofactor MCP, factor H or C4BP. Splicing of C3b by factor I results in the release of a small fragment called C3f and the fragment iC3b which both do not participate in complement activation (1, 3).

And finally, the complement system can also be regulated by controlling the last step of the pathway: formation of the MAC. The membrane protein CD59 prevents the binding of C9 to the C5-8 complex and therefore prevents the formation of the MAC (3). Besides CD59, plasma protein S is also capable of preventing the formation of the MAC by binding to the C7 protein. In this case C8 cannot bind to the complex anymore and the formation of the MAC is stopped (1).

§ 1.6 The receptors of the complement system and the anaphylatoxins

There are several receptors for the complement system, which can occur at different cells. Some will be discussed here. The first receptor is complement receptor 1 (CR1). This is a receptor with a high affinity for C3b or C4b components, and functions mainly to promote phagocytosis of particles opsonised with these complement factors (1). This receptor is mainly found on macrophages, eosinophils, macrophages, monocytes, neutrophils and T and B- lymphocytes.

The second receptor of the complement system is the type 2 complement receptor (CR2). In contrast to the CR1 receptors, this receptor is specific in stimulating humoral immune response. This receptor gets activated by cleavage products of C3b, which are C3d, C3dg and iC3b. As the function of the CR2 receptor already reveals, this receptor is mainly found on B lymphocytes.

Then there is also a type 3 receptor (CR3), also called Mac-1. Like the CR2 receptors, this receptor can be activated by iC3b. The Mac-1 receptor can promote phagocytosis, because it is present on neutrophils and monocytes. However, it can also stimulate recruitment of leukocytes to sites of infection and tissue injury, by binding to intercellular adhesion molecule 1 on endothelial cells (1). Besides that the Mac-1 receptor is present on neutrophils and monocytes, it is also present on mast cells and NK cells.

A receptor with a similar function as the Mac-1 receptor is the type 4 complement receptor (CR4). Like the Mac-1 receptor and the CR3, it binds iC3b. This receptor is mostly expressed on dendritic cells (1).

These were all receptors which are activated by C4 or splicing components of C3. But as mentioned in previous paragraphs, with the splicing of complement components, some small fragments are released. By the splicing of C3, the fragment 3a is released, for C4 this is C4a, and for C5 this is C5a. These small fragments work as anaphylatoxins, which means they can attract immune cells (8). These fragments have their own receptors on specific cells to fulfil their functions (about the receptor for C4 is little know, so it will not be discussed here).
The receptor for C3a (C3aR) is found on eosinophils, dendritic cells, monocytes/macrophages, and mast cells (10). For C5a, there are two receptors, $C5a_1$ receptor ($C5a_1R$) and the $C5a_2$ receptor ($C5a_2R$). The last one is most of the time co-expressed with the $C5a_1R$. They are both found on neutrophils/macrophages, mast cells, immature dendritic cells, and astrocytes (11).
The expression of the receptors can be influenced by for example inflammation. For instance, IFN-y upregulates the C3aR expression, which leads to expression of the C3aR on T-cells, cells where there normally not expressed on (10). The C5a receptors (C5aR) also can be upregulated, the cytokine IL-6 can induce this up regulation, which can occur in different organs, including the kidney (11, 12). Up regulation of these receptors when the complement system is activated leads to more infiltration of immune cells to the site of infection of injury. This will be further discussed in chapter 2.

§ 1.7 Production of complement factors
The classical thought about the production of the complement factors was that they were only made in the liver. However, it has been discovered that complement factors can be produced elsewhere. The liver produces inactive precursor enzymes, which are called zymogens. If these zymogens are activated, they become active protease. These active proteases are capable activating other complement factors by cleavage of the factors (1).
Complement synthesis by extrahepatic sources is most of the time stimulated by cytokines. These extrahepatic sources can be epithelial cells, fibroblasts, lymphocytes and macrophages derived from different organs, including the kidney, according to Fearn and Sheerin (8). The extrahepatic production of complement factors turn out to be contributing to the total circulating C3 for 10% (8). The production of extrahepatic complement factors seems to play a crucial role in local complement activation and local tissue injury (13). An example will be given in paragraph 2.3.

Chapter 2: Complement system in kidney diseases

§ 2.1 Antibodies
The complement system is very important in protecting the body against diseases, but in contrast it can also play a role in causing or aggravating diseases. There are diseases which a characterized by the deposition of antibodies in the body, which leads to complement activation. One of these disease is IgA nephropathy, which is characterized by mesangial IgA deposition (14). This deposition of IgA can activate the alternative complement pathway as well as the lectin complement pathway. The latter one is quite interesting, because normally, it gets activated by MBL, however, it seems that together with an IgA deposition, there is also a MBL deposition in approximately 25% of the patients with IgA nephropathy. This deposition could be the result of serum IgA binding to MBL. Besides the deposition of MBL, these patients also have glomerular deposition of L-ficolin, MASP, and C4d. These factors confirm the suggested activation of the lectin complement pathway (14). The complement activation of either pathways, generates C5b-9, which induces mesangial proliferation and matrix expansion (15). This is possible because besides inducing lysis of the cell, the MAC complex can lead to an influx of calcium into the cell, which results in conformational changes and activation of calmodulin-dependent kinases to drive events in the cell. Cell lysis by the MAC complex can be

countered by ion pump in nucleated cells (16).

Another disease which is caused by a reaction of the complement with auto-antibodies is the anti-glomerular basement antibody (GBM) disease, also known as Goodpasture's syndrome (17). In this disease, the body produces antibodies (IgG) against the basement membrane of the glomerulus. These antibodies bind to the membrane and induce an immune response, including activation of the complement system. Subsequently, the activation of the complement system leads to renal injury by the proinflammatory effect of classical pathway activated C5a and/or cell lysis effect of C5b-9 (17). Very similar to the anti-GBM disease is membranous nephrology. This is a disease in which antibodies are produced specific against the podocytes in the glomerulus of the kidney. The deposition of these antibodies leads to the activation of the complement system (18). However, so far, it is not known which complement system is dominant in this disease. Because this is an antibody-mediated disease, it was assumed that the classical pathway was activated, but the antibodies are mainly IgG4, which do not activate the classical pathway. Some evidence has been found that the alternative and the lectin pathway are activated, but also C1q is found in some cases, which after all gives reason to belief that the classical pathway somehow is part of the complement reaction. To find out which pathway is dominant, more research should be done (19).

Then there are some antibody-mediated diseases, in which the cytoplasm of the neutrophils is the target of the auto-antibodies. These diseases are together called antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). First, it was thought that the complement system did not play a role in these diseases, but recently has become clear that especially the alternative pathway does play a part. This could be due to the activation of the neutrophils by ANCA's, which results in the secretion of properdin, C3 and factor B, all factors of the alternative pathway. Activation of the alternative pathway leads to further activation of neutrophils, and therefore acts as a positive feedback loop (20).

Another way in which the complement system can be the cause of the disease is that the body makes antibodies against complement factors. For example, an auto-antibody against the complement factor C1q. This auto-antibody is often found in patients with the disease systemic lupus erythematosus (SLE) (21). SLE is an autoimmune disorder, which can be caused by different factors, like genetics, environmental factors and immune regulatory factors. This disease is characterized by multisystem inflammation with the generation of auto-antibodies (22). Especially in patients with lupus nephritis (an inflammation in the kidney), auto-antibodies against C1q are present. (21). Because of the antibodies against C1q, the serum levels of C1q are decreased, which leads to a deficiency of the classical pathway (23).
Interestingly, the complement system does not only have a negative role in SLE, it also provides protection against the development of SLE. This is shown in patients with homozygous deficiency of early components of the classical pathway (C1q, C1r, C1s, C4 and C2). These deficiencies are the strongest susceptibility factors of the development of SLE identified up to now in humans, according to Manderson et al. (23).

Auto-antibodies against C1q was one example, other examples are antibodies against factor H of factor I. These are seen in an autoimmune form of atypically hemolytic uremic syndrome (aHUS), a life threatening disease which leads to renal failure due to platelet micro-aggregates in the renal capillarity's (21).

As a last example, there is an auto-antibody that binds the alternative pathway C3 convertase

(C3bBb) and stabilizes it, which prolongs the half life time to minutes or even hours. This auto-antibody is called the C3 nephritic factor (C3NF). When C3NF binds to the C3bBb complex it prevents the intervention of regulator complement factors like factor H, factor I or DAF. Therefore, the C3bBb cannot be dissociate. This and the fact that C3NF stabilizes the C3bBb complex, is the reason why C3NF causes over activation of the alternative complement pathway, which leads to renal injury (24).

§2.2 Genetic deviations

Besides that the complement system can play a role in disease due to antibody interaction, complement can also be the primary reason of disease. In this case, mostly it has to do with genes which are responsible for factors of the complement system.

A disease in which this genetic factor is clearly noticed, if it is not caused by E. coli or auto-antibodies, is aHUS (also discussed in the previous paragraph). In this disease, like in more kidney related diseases, the alternative pathway seems to be of great importance (25).

To induce this disease, a heterozygous loss-of function mutation can occur in, for example, the genes of factor H, factor MCP or factor I (26). A loss-of function mutation in these genes results in an over activated complement system, mainly boosted via the alternative pathway, since these are all genes to control the activation of the complement system. Not only loss-of function can lead to this disease, but also gain-of function mutations in complement factor B or C3, key proteins of the alternative pathway, can play a role in the onset of aHUS (25).

C3 glomerulopathy is an umbrella name for several kidney disease associated with C3 as a critical pathogenic factor. This main group can be divided in two subgroups of diseases: C3G-dense deposit disease and C3 glomerulonephritis. Both of these diseases can have auto-immune causes, but also genetic causes. Genetics factors of these diseases includes mutation in the C3 genes, factor B genes, as well as factor H and the complement factor H related (CFHR) proteins CFHR 1, CFHR2, CFHR3 and CFHR5 (24). Alterations in these genes can, like in aHUS, lead to over activation of the complement system, which will lead to the onset of the diseases.

§2.3 Kidney transplantations

A kidney transplantation is a delicate process, in which many pathways of the immune system play a role, and one of them is the complement system. The complement system plays a role in the humoral allograft kidney rejection, but seems also to play an indirect role in de cell mediated rejection.

Primarily ischemia reperfusion injury (IRI) is a kind of kidney rejection which can be caused by the innate immunity and thus the complement system (4). By IRI, the blood flow in the kidney is disturbed and restored, which provokes an immune reaction (27). As part of this immune reaction, the complement systems gets activated. However, the classical pathway does not seem to be important for the occurrence of IRI, because mice with a C4 deficiency did not seem to have protection against IRI. In contrast, Zou et al. discovered that the MAC plays a critical role in the renal post ischemic injury, because mice with a deficiency in C6 exhibited a similar degree of protection to those with interruption at C3 level (28).

Like the MAC, the complement factor C5a seems to be important in IRI. C5a can attract pro-inflammatory cells like neutrophils. These anaphylatoxins are the bridge between the complement system and the adaptive immune system. They can bind to C5aRs on antigen presenting cells and T-cells to activate them, and therefore can induce cell mediated rejection (4). Because of the effects of C5a on pro-inflammatory cells, it has been studied what the effect will be of a C5a-receptor antagonist in a model for renal IRI. These studies show that an antagonist for the C5a-receptor is capable of attenuate renal injury (29, 30). This implies a role for C5a in IRI and therefore it implies

that it can be a potential therapeutic target, however, targeting the C5aR is likely to be more effective, because it seems that this receptor is up regulated during IRI. This up regulation can be caused by the production of IL-6 of macrophages, which can be initiated by C5a and C3a (31, 32).

For the occurrence of renal failure discussed above, the classical pathway of the complement system did not seem to play a role, however, the classical pathway may play a role in renal rejection in a different way, namely in the antibody-mediated rejection. The antibodies from the donor can activate the classical pathway (4, 17). This will lead to the cleavage of C4, resulting in C4a and C4b. C4b can then be cleaved again in a fragment named C4c and a fragment called C4d. The activation of this pathway will lead a change in endothelial function (8). The last fragment, C4d, binds to the cell membrane and together with histological changes, it is considered as a marker acute humoral rejection (4, 17).

The complement factor C3 appears to have a role in renal rejection as well. As mentioned in paragraph 1.6, complement factors are produced by the liver, but also can be produced by the kidney. Especially this local production of C3 plays a role in renal rejection; it even appears that local synthesises of C3 has a greater influence on rejection than circulating C3 (33). The local C3 can be synthesized by endothelial cells, mesangial cells, glomerular epithelial cells and tubular epithelial cells, of which the last one seems to be the main site (27, 33.). During IRI, the regulatory factor of the complement system seem to be overwhelmed or seem to be lost, according to Damman et al., which leads to an over activation of the complement system and increased local C3 production(27). This will lead to renal injury and eventually contribute to renal rejection.

Not only local synthesis of C3 has a role in renal rejection, donor C3 seems to be of importance too. Kidneys for transplantation can be retrieved from living, heart beating (brain death) or non-heart beating donors (27). It has been discovered that in brain death donors, an immune response is already started in the kidney, where especially C3 levels are increased. Thus, with a transplantation, the kidney of a brain death donor already has more C3 than a kidney of a living donor, which is adversely for emerging of renal injury (27).

§2.4 Progression of renal injury

In the previous paragraphs, the complement system has been described as the causes of renal failure. However, the complement system is not always the cause of the renal failure, but can also be a secondary event. There are many events that can trigger an immune response in the kidneys, which has nothing to do with the complement system at first. For example, unilateral uretic obstruction, an experimental way to induce renal failure in mice. In this example, the formation of the MAC does not seem to have influence on the renal injury, but macrophage infiltration and interstitial fibrosis due to complement component C5 does seem to be important. Namely, mice with a deficiently in C5 had reduced macrophage infiltration and fibrosis and treatment with a C5 receptor antagonist in mice had a protective function against renal failure (8). Thus, in this case, the complement system is not the cause of the injury, but it shows that once the immune system is activated, it plays a significant role in the progression of renal injury. And it is not only the circulating complement that plays a role, especially the local produced complement factors seem to be important in progression of renal injury (13), an example of this was given in paragraph 2.3.

Not mentioned before is that with kidney diseases, often the filtration in the glomerulus is affected, which results in proteinuria, which is a marker for renal damage. Proteinuria can cause activation of the complement system, because renal tubular epithelial cells come in contact with complement

factors. For the complement factors properdin and factor H has been shown that they can bind to the tubular epithelial cells by binding to tubular heparan sulfates. However, they both bind to different epitopes of the heparan sulfate chain. Binding of properdin to tubular heparan sulfate can lead to activation of the alternative complement pathway (this has been discussed in paragraph 1.1), which might play a role in proteinuric renal damage (6, 9).

Chapter 3: Therapies against the complement system

§ 3.1 Standard treatment

Depending on the cause of renal failure/injury treatment modality is chosen. Very comment are angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. These are medication that keep the blood pressure low, which prevents more kidney injury caused by high blood pressure. This slows down the progress of renal failure, but it does not prevent it (34).
When the kidney is almost not working anymore, kidney replacement therapy such as dialysis is needed. During dialysis the patient is attached to a machine which purifies the blood, almost like a good working kidney would do. Patients which are eligible for dialysis are patients with loss of renal function, acute renal injury and end-stage renal disease (35).

For antibody-mediated diseases like Goodpasture's syndrome, plasma exchange is a treatment which can be used. With this treatment the plasma of the blood of the patient is replaced with plasma from a donor or the plasma of the patient is purified. In both cases, the antibodies causing the disease are removed, and in order to prevent the new production of autoantibodies, the patient can be given immunosuppression medication (36). By reducing the amount of pathogenic antibodies, also the binding and activation of complement is reduced.

The treatments mentioned above are treatments which cannot prevent the occurrence of renal failure, they just slow down the process. Therefore there should be more research for other therapeutic intervention options. As mentioned above, the complement system seems to play in many ways an important role in renal injury, therefore it could be a good target for medication. So far, there are two therapeutic interventions that prevent (progression of) renal injury by inhibiting the complement system. One of them is the recently found Eculizumab, and the other one is a C1 inhibitor. Both will be discussed in paragraph 2 and 3, respectively, of this chapter.

§ 3.2 Eculizumab

As mentioned above, eculizumab is a drug that engages with the complement system. To be specific, eculizumab is a humanized antibody against complement factor C5. By binding C5, it prevents splicing by the C5 convertase. Therefore, neither C5b is produced which is the first component for the formation of the MAC, nor C5a anaphylatoxin is formed, thereby preventing chemoattraction of inflammatory cells. Thus, by binding to C5, eculizumab inhibits the formation of the MAC and therefore inhibits renal injury. And because it inhibits C5, a component which is for all complement pathways the same, it does not matter which complement system is activated, eculizumab will still inhibit the formation of the MAC (37).
One disease in which eculizumab is accepted as official treatment by the FDA, is aHUS (previously discussed in paragraph 2.1 and 2.2) (38). Before the acceptance of eculizumab, some clinical trials were done to see the effects and side effects of this medicine. These clinical trials included adults with aHUS resistant to plasma therapy, patients which required chronic plasma therapy, and patients who needed dialysis. In all the trials, eculizumab improved the haematological parameters and the

estimated glomerular filtration rate. Besides this, most of the patients were even able to stop with dialysis or plasma therapy (38). According to Legendre et al. eculizumab inhibited complement-mediated thromobic microangiopathy and was associated with significant time-dependent improvement in renal function in patients with aHUS (39).

Besides the beneficial effects of eculizumab in aHUS, it seems that it also could help in preventing antibody mediated rejection by renal transplantation (40), and it has shown beneficial effects in C3 glomerulopathy (41). Thus so far, eculizumab seems to be a very promising medicine in diseases caused by the complement system.
But, the use of eculizumab also can have some side effects, because in normal situations, the MAC helps protecting the body against infections. Therefore, when eculizumab is used as a treatment, patients are vaccinated especially against *Neisseria* species, which can cause meningitis.
However, because this medicine engages with the complement system very late in the cascade, the earlier complement components, like C3 are still intact, so opsonisation and clearance of immune complexes is still possible (37). A disadvantage of the late engagement in the complement system is, for example, that components like C3a and C4a still can promote the influx of immune cells, which still leads to inflammation and renal injury. The moment of inhibiting the complement system will be discussed more in detail in paragraph 4.1. Another disadvantage of eculizumab is, that it is extremely expensive, preventing wide clinical use, therefore it is important to find drugs which are as effective as eculizumab, but not that expensive (42).

§ 3.3 C1 inhibitors
Another drug which interferes with the complement system and is FDA approved, is the C1 inhibitors (C1-INH). So far, they have only been approved to treat patients with hereditary angioedema, in which it is used to restore the level of function C1-esterase inhibitor in a patient's plasma (43).

Besides using C1-INH to restore a deficiency, it recently has been tested in clinical trials for the inhibiting effect on the complement system in kidney transplantations. Because, as discussed in paragraph 1.5, C1 INH is a natural inhibitor of the classical pathway (1). However, it is also capable of inhibiting the lectin pathway, because of the analogous serine proteases (44).
One example of a clinical trial (phase I/II) in which C1-INH is used, is a trail of Vo et al. In this research C1-INH seems to prevent antibody mediated rejection in patients who had a kidney transplantation (44). Especially with antibody mediated rejection, C1-INH can have great influences, because this rejection is often mediated by the classical pathway (paragraph 2.3).
Besides the use of C1-INH in kidney transplantations, there are no other clinical trials so far for the effect of these inhibitors on other complement related injury in kidneys. The reason for this could be that often the alternative pathway is important in causing the injury, and these inhibitors do not affect the alternative pathway. Therefore it is important that research is done about therapeutics on different levels of the complement system. Some research has already been done in this field and will be discussed in the next chapter.

Chapter 4: New developments of therapies

§ 4.1 Inhibition on different levels
As already briefly discussed in the previous chapter, inhibition of the complement system goes not without some major side effects, like increased risk of infection. To minimalize these side effects, it can be interesting to look at inhibiting the complement system on different levels.

Eculizumab, (chapter 3.2), interferes in the late phase of complement activation, leaving the rest of the pathway functional. In this way, complement functionality is not fully blocked, but there is a beneficial effect.

Another point to inhibit the complement system would be at the level of C3 cleavage. This could for example be more efficient for patients with C3 glomerulopathy than inhibition with eculizumab (4). It depends on how the C3 cleavage is inhibited what the side effects are. If it is inhibited by preventing the formation of the C3 convertase (C4b2a), the function of opsonisation and clearance of immune complexes of the complement system is lost. Therefore, patients almost lose their complete humoral innate immune system, which make them vulnerable for diseases.
However, the alternative pathway has a different C3 convertase and it 'creates' its own C3b by a tick over process, so this pathway will still be activated. To make sure that neither of the pathways is working, an antibody should be created that inhibits the cleavage of C3 by binding to it. It also should prevent tick over of C3. Although this might be possible, this would probably not be very beneficial because inhibition of all pathways makes patients very vulnerable for diseases. However, this problem could possibly be resolved by vaccination and long term use of antibiotics (45).

These example, inhibiting at C5 level or at C3 level, already show that inhibiting the complement system on different levels can have different effects. Because the complement system is a very complex system, it is of great importance to inhibit the system very specific. This could be for example by influencing the regulator proteins of the complement system, which is already examined in some research. The next paragraphs will briefly discus C3 convertase inhibitors, inhibitors of regulation factors, receptor antagonists and polysaccharides.

§ 4.2 Compstatin and Cp40
Compstatin is an inhibitor of the complement system, this cyclic peptide can bind to C3 and can interfere with convertase formation and C3 cleavage (45). So far, compstatin has not been tested in the clinic. However, an analog of compstatin called Cp40 has shown, in vitro, to be beneficial for patients with C3 glomerulopathy (46). If this effect returns in a clinical test, this could be a new medicine for treatment of complement related diseases. But, as discussed earlier, inhibiting of C3 does not go without side effects, and so far, nothing is known about how severe the side effects will be (46). There are plans for testing Cp40 (AMY-101; Amyndas Pharmaceuticals) in a clinical trial in 2015, but till now, no clinical trial is found (47).

§ 4.3 Properdin
Properdin is the only positive regulator of the complement system and only plays a role in the alternative pathway, it stabilizes the alternative pathway C3 convertase. This could be a great target for therapy because the alternative pathway plays a major role in many diseases. But, so far, there is not a specific medicine that targets properdin. Recently, however, some research has been done about antibodies which bind to properdin and inhibit its function. One of these studies is a study of Pauly et al. (48). In this study they used a mouse monoclonal antibody (mAb) 1340 against human properdin. It turned out (in vitro) that this mAb was very successful in inhibiting the alternative pathway, especially the alternative pathway mediated cell lysis. Therefore, mAb 1340 could be a potential properdin inhibitor (48). But before this can be used in a clinical trial, further research should be done, for example to find out which side effects it will have and if it should be blocked in the circulation or local (49) and how it is possible to block properdin locally.

Besides mAb's, there seem to be some other ways to inhibit properdin. As previously discussed in

paragraph 2.4, properdin can activate the alternative pathway by binding to HS. Thus, by preventing the binding between properdin and HS, activation of the alternative pathway, in this way, can be prevented, which could reduce renal damage. Preventing this binding seems to be possible with nonanticoagulant heparinoids (6).
Another way of inhibiting the activation of the alternative pathway was found in tick saliva, which consists of many proteins that are capable of inhibiting the alternative pathway by dissociating the components of the C3 convertase, C3b, and cleaved factor B (Bb). One of these proteins is Salp20. Salp20 works by directly binding to properdin, which leads to the release of properdin from the alternative C3 convertase complex. This results in an accelerating decay of the convertase and thus of inhibition of the alternative pathway (50). Therefore, Salp20, like nonanticoagulent heparinoids, can be interesting for future therapeutic intervention.

§ 4.4 Receptor antagonists
The therapeutic targets mentioned so far, are all against complement factors, but the complement system also functions with receptors. These receptors can be blocked, which was already mentioned in paragraph 2.3. Some antagonist are already known, examples are ADC-1004, Anti-C5aR-151, PMX52 and CCX168 (3). The last one will be discussed a bit more. CCX168 is a small molecule antagonist which binds on the C5aR. A study done with this antagonist in mouse with AAV, showed beneficial effects (51). CCX168 is also already tested in a clinical trial with subjects with ANCA-associated renal vasculitis, however, the trial is still going, so no results have been found yet. Further, this antagonist is going to be used in two open-label phase 2 clinical trials. The first one is to evaluate safety and efficacy in subjects with IgA Nephropathy on stable RAAS blockade, which will be done later on in 2015 (52). The second one is to study the effects of CCX168 in aHUS dialysis patients (53).
For the future, it will be interesting to study what the effect of CCX168 will be in humans who had a kidney transplantation, because blockage of the C5aR seem to protect transplant kidneys against IRI in rats (30).

§ 4.5 Polysaccharide glycosaminoglycan (GAG)
GAG's can be divided into four groups, one of these groups are HS's. HS can be a target for inhibiting the alternative pathway, as mentioned in paragraph 2.4 and 4.3. However, GAG's can intervene with the complement system in many different ways and not only with the alternative pathway. HS has shown to interact with a couple of components of the classical pathway, like C1, C1 inhibitor and C4b, which all lead to inhibiting this pathway. For the lectin pathway there is not that much know, but it seems that heparin in combination with anticoagulant antithrombin can bind to MASP-1 and MASP-2, which results in inhibiting these proteases (54).
For the alternative pathway, besides interaction with properdin, the binding site for factor B can be blocked by fluid-phase heparin (54). And, the other specific factor for the alternative pathway, factor H, is also affected by heparin. The interaction between these two components, results in an increase of the affinity for C3b (54).
Besides that GAG's can influence the complement system by interaction with the components, they can also have effect by interaction with the receptors of the complement system. Heparin can act as a competitive inhibitor of iC3b on the CR3 and CR4, which inhibits phagocytosis and recruitment of immune cells (1, 54). Because GAG's seem to intervene with many parts of the complement system, this could be a potential target for drugs.

Conclusion

What have become clear is that the complement system indeed plays an important role in kidney diseases or injury. The complement system can play a role in disease in different ways, it can has something to do with auto-antibodies, with anti-bodies against complement factors, genetic alterations, or it can promote progression of kidney damage (4, 18, 21, 24). However, most of the time, especially the alternative pathway is the problem of the disease (25).
Because the complement system is so important in renal diseases, it is important to have appropriate drugs. One of these drugs is eculizumab, although, this drug is function well, it is too expensive for wide clinical use. Also, it is only inhibiting at the C5 level, so late in the pathway and in all pathways (42, 43). For treatment of kidney diseases it would be more interesting to have a drug that acts in the alternative pathway, because this is the most important one in these disease, and also because this pathway works as an amplification factor for the other pathways (1).
Therefore it is good that research has been done for inhibiting properdin, a specific regulator for the alternative pathway. This seems promising, although it is not in the clinic yet (48). An advantage of only inhibiting properdin is that the other pathways stay intact, and so loss of protection against microbes is minimal. The loss of protection against microbes could also be minimal by inhibiting specific receptors of the complement system. Opsonisation stays intact this way.
Besides these promising interventions, GAG's also seem like a good target for intervention with the complement system, because of the intervention of GAG's in many ways with the complement system (54). But, a lot of research still need to be done in this upcoming area.
Overall can be said that research for drugs for the complement system should focus on specific targets, like properdin and complement receptors, to minimalize the side effects and to be as effective as possible.

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