C1q and anti-C1q autoantibodies in (auto)immunity

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CHAPTER 2

Complement activation and regulation in rheumatic disease

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Abstract

Complement is a key component of the innate immune defense and in addition forms a bridge to the adaptive immune responses. As such complement is of vital importance for efficient protection against infections. However, the activity of the complement system can also aberrantly be directed against the tissues of the body itself and contribute to organ damage in a variety of diseases. In several rheumatic diseases complement activation is suggested to play a pronounced role. This review will highlight the role of both complement activation and complement regulation in rheumatic disease. A contribution of complement to the disease process is often suggested based on the presence of complement activation fragments in the target tissues or the presence of complement activation fragments in the circulation. The role that complement plays in different rheumatic diseases is often unknown but is thought to contribute to tissue damage as a consequence of autoantibody mediated immune complex formation and deposition. In addition reduced complement inhibition mediated by endogenous complement regulators can also enhance complement activity and tissue damage. In observational studies, it is difficult to distinguish whether complement activation is a result of enhanced activation or decreased regulation. Until recently, strong conclusions on the relative importance of complement activation to the pathology were largely restricted to animal experiments. Usage of complement targeting therapeutics in humans will hopefully give us the opportunity to study the actual contribution of complement activation towards disease progression and tissue damage in rheumatic disease into more detail.

Complement activation and regulation

The complement system is one of the oldest parts of the innate immune system and is involved in numerous processes like elimination of microbes, clearance of immune complexes, tissue regeneration and angiogenesis [1]. Unfortunately, while complement is very efficient in protecting against infections, it is also activated against one's own tissue in several autoimmune and rheumatic diseases. Three pathways lead to activation of the complement cascade; the classical pathway, lectin pathway and alternative pathway (Figure 1). All three activation pathways eventually lead to the cleavage of C3 and deposition of C3b, followed by the terminal pathway that results in the formation of the membrane-attack complex (MAC) and the release of anaphylatoxins C3a and C5a.

The classical pathway (CP) of complement activation is initiated when the recognition molecule C1q binds to its ligands such as surface-bound IgM, multimers of IgG, C-reactive protein (CRP), DNA or dead cells [2]. Following the binding of C1q to its targets, the C1 associated enzymes C1r and C1s become active enabling them to initiate complement activity by cleaving C4 and C2, resulting in the formation of the CP C3-convertase C4b2a [3].

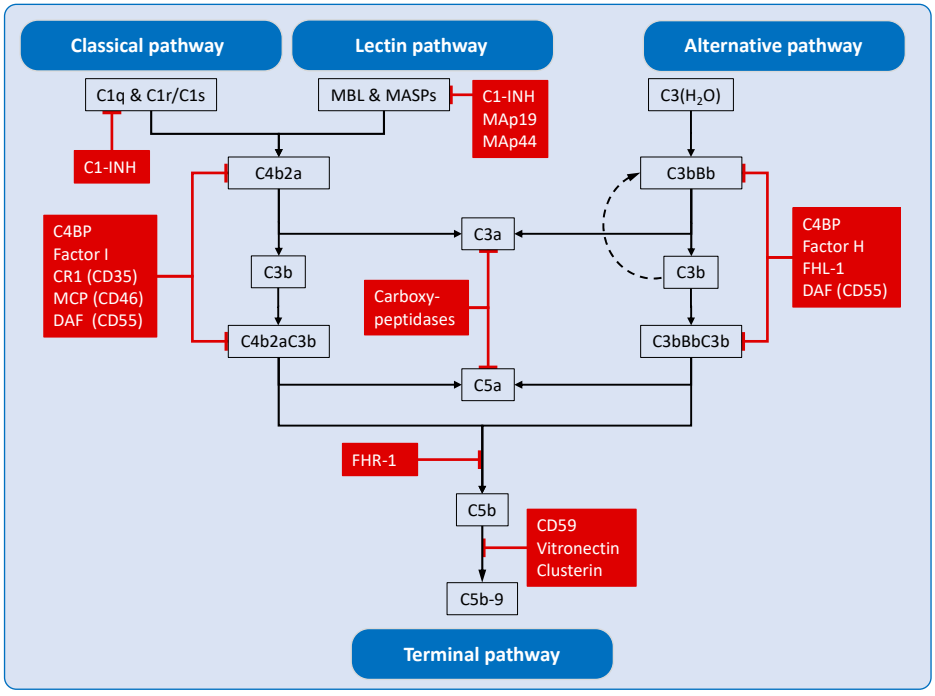

Figure 1. An overview of the complement system, indicating the interactions of activating (black boxes and lines) and regulating (red boxes and lines) complement components.

The lectin pathway (LP) of complement activation is initiated in a similar manner, but by other pattern-recognition receptors (PPRs), like mannose-binding lectin (MBL), ficolins (lectins containing a fibrinogen-like and a collagen-like domain) or collectins (collagenous C-type lectins) [4]. After ligand binding, these recognition molecules activate the LP via the MBL-associated serine proteases (MASPs), which can either activate C4 and C2, leading to the formation of the LP and CP C3-convertase (C4b2a), or directly cleave and activate C3 [5].

The alternative pathway (AP) of complement has a different mechanism of activation. The AP is continuously activated in a spontaneous manner when a small part of the C3 molecules becomes hydrolyzed forming C3(H_2O). C3(H_2O) is bound by factor B and becomes a target for factor D mediated cleavage of factor B. Subsequently, the complex can cleave additional C3 molecules into C3a and C3b. The formed C3b molecules also bind factor B. The subsequent cleavage of bound factor B by factor D forms the AP C3-convertase C3bBb. This process is constantly counteracted on cell surfaces by the endogenous complement inhibitor factor H, which binds polyanionic residues like sialic acid groups and glycosaminoglycans present for instance on human cells. Pathogens often contain fewer of these polyanions, leading to less factor H binding and more complement activation [6]. As such, factor H can be considered the recognition molecule of the AP. The AP is also considered an amplification pathway, as it can be initiated by C3b formed by any of the three pathways [7].

Following the formation of the C3 convertases, the remainder of the complement activation cascade, the terminal pathway, is similar. The C3-convertases form C5-convertases that can cleave C5 into C5b and the potent anaphylatoxin C5a. C5b will, together with C6, C7, C8 and multiple molecules of C9, form the membrane attack complex (MAC) which can cause cell lysis [8]. Most of the circulating complement proteins are produced by hepatocytes, but expression of complement proteins is also recorded for other cell types including leukocytes [9]. Production of several key complement proteins is largely restricted to specialized cell types, for example, factor D is mainly produced by adipocytes and C1q is produced by cells of the monocytic lineage such as macrophages, dendritic cells and mast cells [10-13].

Table 1. An overview of complement regulators, their specific function and references about their role in rheumatic diseases that were consulted in this review.

Name	Full name	Function	References in this review
C1-INH	C1 esterase inhibitor	Inactivates serine proteases C1r, C1s and MASPs	SLE: [110] RA: [70]
MAp19	MBL Associated protein of 19kDa	Competes with MASPs for MBL binding	SLE: [95]
MAp44	MBL Associated protein of 44kDa	Inhibits C4 deposition by binding lectin pathway initiators	RA: [72] SLE: [95]
C4BP	C4b-Binding Protein	Inhibits functions of C4, accelerates decay of C3 convertases	RA: [66, 71] SLE: [28]
Factor H		Binds self surfaces, cofactor for factor I, accelerates decay of convertases	RA: [55-60] SLE: [28, 59, 118, 120, 126] AAV: [137-140]
Factor I		Inactivates C4b and C3b by cleavage, but only after cofactor binding	SLE: [117]
FHL-1	Factor H-like protein 1	Cofactor for factor I, accelerates decay of convertases	RA: [55, 56]
FHR-1	Factor H-related protein 1	Binds self surfaces, inhibits C5 activation and MAC formation	SLE: [119]
CR1 (CD35)	Complement Receptor 1	Cofactor for factor I among other functions (membrane-bound)	SLE: [111, 112]
MCP (CD46)	Membrane Cofactor Protein	Cofactor for factor I (membrane-bound)	SLE: [115, 116]
DAF (CD55)	Decay Accelerating Factor	Accelerates decay of convertases (membrane-bound)	RA: [74-76, 91] SLE: [113, 114]
CD59		Binds C8 and C9, inhibits MAC formation (membrane-bound)	SLE: [113, 114]
Vitronectin		Binds C5b-9, prevents MAC formation	SLE: [80]
Clusterin		Binds C7, C8, C9, prevents MAC formation	SLE: [80]
Carboxypeptidases		Cleaves C3a and C5a to des-arginated forms	RA: [73]

This potentially aggressive and self-amplifying cascade of inflammatory and cytotoxic mediators needs to be tightly regulated to ensure that complement activation is limited in time and location [14]. Excessive and unrestricted complement activation would lead to organ damage and enhanced risk for infections due to the complete consumption of complement. The complement system is therefore regulated by membrane-bound as well as soluble factors (Table 1). There are four general functions of complement inhibitors: some inhibitors bind complement proteins and prevent their incorporation into complement complexes, others bind and terminally inactivate enzymes, other inhibitors serve as a co-factor for enzymatic degradation of complement proteins and other complement inhibitors enhance the decay of C3-convertases [15].

C1-inhibitor (C1-INH) inhibits the classical and lectin pathway by binding and inactivating C1s, C1r and MASPs, whereas MAp19 and MAp44 inhibit the lectin pathway specifically [16, 17]. C4b-Binding Protein (C4BP) inhibits the activity of C4 and accelerates the decay of the C3 convertases of all three pathways [18]. Decay of C3 convertases is also stimulated by factor H, which in addition serves as a cofactor for factor I mediated degradation of C3b [6]. Factor I inactivates C4b and C3b by cleaving them, but only when they are first bound by a cofactor of factor I, such as factor H, factor H like protein 1 (FHL-1), C4BP, MCP (CD46) and complement receptor 1 (CD35). In the terminal pathway, clusterin and vitronectin bind the proteins that form the C5b-9 complex and prevent its completion or insertion into the membrane. A similar function is attributed to CD59 expressed on the cell surface, which binds C8 and C9 to hinder the assembly of the MAC. Another membrane-bound complement regulating protein, decay-accelerating factor (DAF; CD55), increases the degradation of convertases on the cell membrane. Lastly, carboxypeptidase-N converts the potent anaphylatoxins C3a and C5a into their less active, des-arginated forms.

Complement regulation also plays a role in the process of cell death by apoptosis, a massively occurring physiological process required for tissue homeostasis. The clearance of apoptotic cells is a highly efficient process and is suggested to result in an anti-inflammatory or tolerizing effect. However, in many clinical conditions, including rheumatic diseases, the process of clearing dying and dead cells is overwhelmed by either too much cell death or too little clearance. Complement plays an important role in the recognition and efficient phagocytosis of apoptotic and necrotic cells [19]. A complement system malfunctioning as a consequence of genetic, acquired or therapy-induced deficiencies can thus impact the clearance efficiency and cause accumulation of autoantigens, which are released from uncleared dead or dying cells. Several complement proteins have the capacity to bind to dead cells, prominently C1q, but also

MBL, ficolins, collectins, and properdin [20-25]. These complement proteins bind a wide variety of exposed ligands on dead cells [26]. Interestingly, some cells downregulate the expression of membrane bound complement inhibitors, CD46, CD55 and CD59 during apoptosis, potentially leaving the cells vulnerable for complement attack [27]. However, while cells decrease the expression of membrane bound complement inhibitors, they gain the capacity to bind the fluid phase complement inhibitors factor H and C4BP [28]. A wide set of ligands can mediate such binding, but factor H and C4BP can also directly bind to exposed DNA [29].

Complement in rheumatic disease

The complement system is an important factor in host defense, but defects leading to uncontrolled complement activation can cause a variety of clinical conditions. Here, we focus on three major rheumatic diseases in which complement plays an important role and for which complement inhibitory therapy was recently initiated in therapeutic trials: rheumatoid arthritis, systemic lupus erythematosus (SLE) and ANCA-associated vasculitis (AAV). We describe the current knowledge on the influence of complement activation and regulation within these diseases, both endogenous and therapy-induced.

Rheumatoid arthritis & complement

Rheumatoid arthritis (RA) is a chronic inflammatory disease, which affects up to 1% of the population [30, 31]. The main symptoms are located primarily in the small peripheral joints, where chronic inflammation causes damage to cartilage and bone through mechanisms involving both the innate and adaptive parts of the immune system. The lining of the joint, the synovium, is infiltrated by both T and B lymphocytes, as well as macrophages [32, 33]. Later in disease progression, the cartilage and bone in the joint are invaded from the synovium during the formation of a ‘pannus’, causing damage through resorption and breakdown of bone by osteoclasts [34]. The release of proteolytic enzymes like cathepsins and metalloproteinases also lead to the breakdown of matrix proteins in cartilage and bone [35].

Apart from this cellular immune involvement, humoral immunity also plays a major role in RA. Autoantibodies are estimated to be present in 50% to 80% of RA patients [36]. Prominent among these autoantibodies are rheumatoid factor, anti-collagen type II antibodies and antibodies against post-translationally modified proteins, such as anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein (anti-CarP) [37-40]. The autoantibodies present in RA patients often target antigens in cartilage and the

synovium, contributing to the formation of immune complexes. Crucially, these immune complexes can activate complement, which gives rise to chronic destruction of the joint, via the initiation of innate as well as adaptive immune responses. Indeed, it was shown that ACPA can activate complement via both the CP and AP [41].

A possible role for complement in RA was initially concluded from studies that found activated or cleaved complement components in the synovial fluid [42-47]. Later, signs of complement activation, such as C1q-C4 complexes, were also identified in the circulation of RA patients [48]. The activating role of complement in RA is illustrated in collagen induced arthritis (CIA) models, where mice with a compromised complement system are less affected by the disease [49-51]. Currently, it is still not completely known which ligands are triggering this complement activation.

Several animal models for arthritis are regularly used to study pathogenic mechanisms. Several studies in animal models of arthritis, such as the collagen antibody-induced arthritis (CAIA) model, revealed that exclusively the alternative pathway is essential for disease development [52-54]. Although the classical pathway does contribute to disease, it was not essential in these models. The large role of the alternative pathway in RA makes research into its regulators very interesting. Factor H and its variant, factor H-like protein 1 (FHL-1), may have a protective role against complement damage in the synovium [55]. Both proteins are expressed by synovial fibroblasts from RA patients and their production was increased after stimulation with either IFN-γ or dexamethasone [56]. Intervention with recombinant complement receptor 2 linked factor H (CR2-FH) in a CAIA model showed decreased disease activity and C3 deposition in the synovium [57]. Another study in the same model showed that complement activation was increased when the tissue binding domain of factor H was blocked, providing more evidence that factor H plays a vital role against arthritis in this mouse model [58]. Additionally, autoantibodies against factor H were found with higher frequency in RA patients compared to healthy controls [59]. Single nucleotide polymorphisms (SNPs) of factor H known for their association to macular degeneration, were however not found to correlate with development of RA [60].

RA joints may contain apoptotic and necrotic cells due to prior damage, which can trigger complement activation [20]. Furthermore, complement can be activated by proteins originating from the extracellular matrix (ECM) of damaged cartilage in the joint, like osteoadherin, fibromodulin, aggrecan and cartilage oligomeric matrix protein (COMP) [61-64]. For example, levels of COMP-C3b complexes were found to be elevated in RA patients compared to healthy controls [65]. Several ECM proteins also bind C4BP and

specific ECM proteins with inhibitory properties, such as decorin, have been described as well [61, 66, 67]. Due to complement activation on proteins from the ECM, RA patients often have increased concentrations of complement activation fragments and show signs of higher C4 and C3 consumption in the synovium. Complement activation by immune complexes and other protein triggers is thought to be a mediator of inflammation, attracting effector cells mainly through the anaphylatoxin C5a, again stimulating the inflammatory state in the joint [68]. Another consequence of complement activation in the joint is the direct stimulation of collagenase production in synovial fibroblasts by sublytic levels of the membrane attack complex [69].

Multiple complement proteins, such as C1q and C1-INH, are also synthesized locally in the synovium next to their systemic production, suggesting a local role [70]. Other fluid phase regulators were also investigated: In a CIA model, mice that were given human C4BP showed later disease onset. When C4BP was given only after onset, mice showed reduced disease severity [71]. The lectin pathway regulator MAp44 also plays a role in limiting complement activation in a CAIA model in mice [72]. In this study, adenovirus programmed to express human MAp44 was injected into mice, which resulted in a significant reduction of the disease activity score. Deficiency of another fluid phase regulator, carboxypeptidase B, was found to aggravate arthritis in a mouse model [73]. Moreover, an allele encoding a carboxypeptidase B variant with longer half-life was linked to a decreased risk of developing severe disease in RA patients [73].

The membrane bound complement regulator CD55, also known as decay-accelerating factor (DAF), is an important factor that protects cells from complement attack by dissociating C3 and C5 convertases. Expression of CD55 on fibroblast-like synoviocytes is actually found to be high compared to leukocytes and endothelial cells [74]. Next to a role in complement, CD55 can also act as a ligand for CD97. CD97 is expressed on a variety of cells including infiltrating macrophages and T cells, and may thereby contribute to continued inflammation in the joint via other mechanisms as well [75, 76]. These results indicate that CD55 both regulates complement activation in the joint and contributes to immune cell infiltration and inflammation.

Since the animal models suggest a crucial role of complement in the disease process of RA, intervention studies with complement inhibitors have also been performed in RA. Surprisingly, despite the observed complement activation in human joints and the compelling evidence in mice, there was no benefit in RA of blocking the C5a – C5a receptor interaction [77]. Studies using eculizumab to block cleavage of C5 were not

sufficiently beneficial for further trials either. Consequently, there is no obvious major role for complement therapeutics reported so far in RA, highlighting the disparity between animal models and patients.

Systemic lupus erythematosus & complement

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease that affects between 0.01% and 0.1% of the population. SLE mainly affects women around the child bearing age. SLE is a systemic disease where many organs are affected, either simultaneously or consecutively. Organs affected in this disease include, but are not limited to, the skin, joints, kidney and brain. Especially the involvement of the kidney, called lupus nephritis, is a major contributor to morbidity. The recent focus on brain involvement in lupus also increased the awareness of neuropsychiatric complications [78]. SLE is characterized by the generation of a large variety of autoantibodies (reviewed in [79]). The hallmark autoantibodies in SLE are generated against moieties that are normally not exposed outside the cell, such as double stranded DNA and nuclear proteins. The autoantibodies characteristic of SLE form immune complexes with these nuclear components when they are released from dead cells. Most symptoms of SLE are thought to result from immune complexes formed by these autoantibodies, which deposit in tissue and subsequently cause an inflammatory response by binding complement and Fc receptors. Circulating immune complexes activate complement too, although clusterin and vitronectin were found to offer some protection by their presence on circulating immune complexes containing precursors to MAC [80]. First evidence of complement involvement in SLE came from findings of complement deposition in affected organs, followed later by observations of decreased levels of complement factors and increased levels of complement activation fragments were found [81-83].

The role of complement in SLE is two-fold. On the one hand, complement activation is important for inflammation and tissue damage in most patients. On the other hand, rare genetic deficiencies of several complement components predispose for development of SLE [84]. Deficiencies in the classical pathway give a high risk of developing SLE, with deficiency of C1q causing the highest risk [85]. Several of such C1q deficient patients have been described [86-88]. Noticeably, there is a wide heterogeneity in the clinical presentation and severity of disease in these patients [89, 90]. Especially in C1q deficient SLE patients, a high proportion of patients experience neurological problems, which may be related to a role for C1q in shaping or maintaining nerve interactions [86, 91]. Why C1q deficient patients have such a high risk of developing SLE is currently not certain. Several mechanisms have been suggested, including the reduced clearance of

apoptotic cells and the reduced suppression of T cell activity [92, 93]. While apoptotic cells are generally not detected in vivo because of their efficient clearance, they were detected in C1q deficient patients, suggesting slower clearance. C1q binds the surface of apoptotic cells and enhances phagocytosis, as discussed in the section Complement activation and regulation. For a strong autoantibody response, T cell activity is required. The reported function of C1q as an inhibitor of T cells responses could fit with the notion that in C1q deficiency T cell help is provided to autoreactive B cells [93]. However, both proposed mechanisms may be insufficient to fully explain the risk to develop SLE and other mechanisms may contribute as well.

The lectin pathway has also been studied in detail in SLE, revealing that the levels of several lectin pathway proteins correlate with the (decreased) levels of C3, which suggests consumption because of activation [94, 95]. Meta-analyses on mutations associated with MBL deficiency indicated an modestly increased risk for SLE in MBL deficient individuals [96, 97]. Furthermore, animal models of SLE suggest activation of the lectin pathway during the course of disease [98]. The serum levels of lectin pathway inhibitors Map44 was reported to be slightly higher and MAp19 to be slightly lower in SLE as compared to healthy controls [95].

Autoantibodies have been described for nearly all complement proteins [99]. In the context of SLE, especially anti-C1q, anti-MBL and anti-ficolin-3 are important [100-102]. Autoantibodies against C1q are associated with lupus nephritis in SLE patients, although anti-C1q antibodies are also present in a small part of the healthy population without signs of renal problems [103, 104]. Mouse studies have shown that the contribution of anti-C1q autoantibodies to lupus nephritis is dependent on the prior presence of C1q binding immune complexes in the glomeruli [105, 106]. For anti-ficolin antibodies an association with lupus nephritis was also reported, while for the anti-MBL such association was not observed [101, 102]. The functional consequences of anti-MBL autoantibodies to the pathogenesis is currently unclear [101, 107-109]. Antibodies against soluble regulators were also found. For example, anti-C1-INH autoantibodies were found to be increased in SLE patients and were associated with high disease activity scores [110]. Factor H antibodies were also found in SLE patients, but not at a significantly higher rate than in healthy controls [59].

An important player in the clearance of immune complexes, complement receptor 1 (CR1), is found on several types of myeloid cells, including monocytes, dendritic cells and erythrocytes. CR1 binds targets opsonized by C4b and C3b, and is involved in the transport and uptake of immune complexes. Erythrocytes of SLE patients were found to have decreased levels of

CR1, while stimulation of erythropoiesis in lupus patients with anemia resulted in improved CR1 function [111, 112]. As a result of reduced CR1, these patients cannot clear new immune complexes as effectively and their erythrocytes are more vulnerable to complement attack, because they have lower complement inhibitory activity of CR1. This may result in a vicious circle that conserves a disease state that is hard to treat.

Membrane-bound complement regulatory proteins are important to protect cells from complement activation. Decreased levels of CD55 and CD59 were found on lymphocytes in SLE patients, which may contribute to lymphopenia in these individuals [113, 114]. In serum of patients with active SLE, increased levels of soluble CD46 were found, compared to healthy controls or patients with other autoimmune diseases [115]. The higher levels soluble CD46 may be explained by increased CD46 shedding by T helper 1 cells, which is related to hyperactivity and defective contraction in these cells [116].

Soluble regulators are naturally of great importance as well, and their disruption may cause serious complement damage. Factor I levels in SLE patients were found to be negatively correlated with disease activity score [117]. Some SLE patients with lupus nephritis were described to have dysfunctional factor H. Purified factor H from 3 out of 4 patients induced less phagocytosis of late apoptotic cells and protection against lysis of erythrocytes was decreased in 2 of the patients [118]. Factor H, as well as C4BP, is also important in the protection of apoptotic cells against uncontrolled complement activation and lysis [28]. A large case-control study on factor H and factor H-related genes concluded that a CFHR3-1 deletion was associated with SLE and might contribute to development of the disease [119]. The impact of factor H is underlined in an animal study, where factor H deficient MRL-lpr mice showed accelerated development of lupus nephritis compared to factor H sufficient mice [120].

In view of the broad involvement of complement in SLE, it seems evident that therapeutic targeting of complement in SLE patients could be beneficial. However, complement targeted therapy has so far only been used sporadically in SLE. Genetic deficiencies of classical pathway components were successfully overcome by repeated plasma infusions, which resulted in disappearance of SLE symptoms for weeks after infusion [88, 121]. Transplantation of hematopoietic stem cells has also been tested in C1q deficient individuals; clinical results were good for two patients, while a third did not survive [87, 122]. Eculizumab has been used in a few patients where excessive complement activation contributes to disease, with good results [123, 124]. Another recent study investigated the treatment of SLE by mesenchymal stem cell (MSC) transplantation, obtaining either a major or partial clinical response in the majority of patients [125].

This clinical effect of MSC transplantation was reportedly due to increased factor H production [126]. However, more studies will be needed before complement-targeting therapy could be applied on a large scale in SLE patients.

ANCA-associated vasculitis & complement

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a disease that covers multiple clinical conditions including granulomatosis with polyangiitis (GPA), eosinophilic GPA and microscopic polyangiitis. These diseases have in common that the vast majority of patients (90%) are positive for ANCA [127]. Two different neutrophil proteins are recognized as targets for ANCA: proteinase 3 (PR3) and myeloperoxidase (MPO) [128, 129]. In a resting state, these targets are only found intracellularly, but priming through complement activation fragment or cytokine-mediated stimulation causes the ANCA antigens to also be expressed on the cell surface. The exposed ANCA antigens can now be recognized by the antibodies. Neutrophils are activated by ANCA binding, resulting in degranulation and migration into the wall of the blood vessel, thereby contributing to vasculitis [130]. The full pathogenesis of AAV has recently been reviewed [131]. Renal involvement in AAV is characterized by pauci-immune glomerulonephritis [132]. The histological lesions in the kidney are characterized by crescents and fibrinoid necrosis in the absence of a pronounced presence of immune complexes or complement [127]. Because of this near absence of complement activation staining in the kidney and the absence of hypocomplementemia, no role for complement was anticipated at first. This view changed when a role for complement in AAV was shown in mouse models: the development of ANCA-induced glomerulonephritis was shown to be dependent on alternative pathway activation and C5a signaling [133].

Next to the neutrophil activation by C5a, complement also plays an amplifying role after the initial neutrophil activation. Binding of ANCA to its antigen on the neutrophil can give rise to a range of actions, such as degranulation, neutrophil extracellular trap (NET) formation and respiratory bursts. Neutrophil activation can also cause novel complement activation. One study found that complement in human serum is activated upon addition of supernatant from ANCA-stimulated neutrophils, but not by supernatant of unstimulated neutrophils, measured at the level of C5a [134]. Consequently, there is a local increase in leukocyte infiltration, neutrophil activation and tissue damage, again continuing the inflammatory state [135]. The secondary complement activation seems to be mediated through the alternative pathway. In mouse models of vasculitis induced by MPO-ANCA, C4 deficient mice (lacking classical and lection pathway activation) were susceptible to development of glomerulonephritis and vasculitis, while animals

deficient for alternative pathway activation were protected from the disease [133]. How this alternative pathway activation is triggered remains unclear, but activation of the alternative pathway by activated platelets was recently suggested [136].

As the main regulator of the alternative pathway, factor H could be an important player in limiting secondary complement activation. Indeed, plasma factor H levels were significantly lower in patients with active AAV as compared to AAV patients in remission and healthy controls [137]. Further research from the same group showed that factor H from AAV patients is often less active in binding and regulating C3b and in guarding self-cells against complement damage [138]. Neutrophils activated by ANCA can release myeloperoxidase, which in turn can bind factor H and inhibit the interaction between factor H and C3b [139]. Interestingly, normal factor H binds to neutrophils where it can inhibit ANCA-induced activation and degranulation. In a coculture system of neutrophils and human glomerular endothelial cells, addition of factor H increased migration of neutrophils towards the endothelial cells and decreased activation of and damage to endothelial cells [140]. In the same study, it was found that factor H from AAV patients was dysfunctional in the binding to neutrophils, indicating a role for factor H in the pathogenesis of AAV.

Despite involvement of multiple complement factors in AAV, C5 remains a key player [134, 141]. Recently, blocking of the C5a receptor (C5aR) was shown to protect mice against ANCA induced glomerulonephritis [142]. In this study, C5aR deficiency ameliorated the effect of induced glomerulonephritis, while animals with human C5aR showed similar disease as wildtype mice. Importantly, the human C5aR inhibitor CCX168 was used to block human C5aR in mice, resulting in drastically less necrosis and crescents in the glomeruli and lower neutrophil infiltration in the glomeruli [142]. Subsequently, a Phase 1 clinical trial was started, which showed that CCX168 (now also called avacopan) was tolerated in humans without major issues [143]. Further studies where CCX168 partially or fully replaced the use of prednisone in AAV patients displayed effectiveness at least similar to the current standard treatment for these patients in terms of nephritis inhibition [144]. These promising trails could open the door to novel complement-directed therapy in the field of rheumatic diseases where, as for AAV, initially a role for complement was not anticipated.

Discussion

The complement system is important in many physiological processes, but can also be a mediator of disease. The involvement of complement in rheumatic diseases is often deduced from the presence of complement deposition in affected tissue and/or increased levels of complement activation fragments. This does not necessarily mean

that complement plays a causal role in each of the rheumatic diseases discussed here. Present literature is largely focused on the activating ligands and complement factors as opposed to complement regulators. However, it is not always clear whether aberrant complement activity is the result of excess activation or reduced regulation. Especially in RA and SLE, a reasonable number of complement components have been related to the disease in one way or another. Nevertheless, for many it remains unknown whether these associations could be used as predictive biomarkers or therapeutic targets. This is a theme that could definitely benefit from deeper investigation in the future. In AAV, the key complement component that contributes to disease is C5, acting mainly through C5a signaling, with a promising C5aR inhibitor currently being studied in clinical trial. Such complement targeting therapy seems less likely for RA in the near future, since several other treatment options are already available. The balance between activation and regulation of complement seems particularly important in SLE, where reduced complement activation can impede clearance of potential sources of autoantigens like apoptotic cells, while increased activation on deposited immune complexes contributes to tissue damage. Although a small number of SLE patients has been treated with eculizumab with good results, a broader investigation is needed before this therapy could be applied on a larger scale. A key contribution in complement-targeting therapy could be made by natural complement regulators or mutant variants thereof. Another interesting feature of future complement therapy may be locally targeted regulation instead of systemic inhibition. This gives the advantage that the complement system is still capable of functioning normally in nonaffected parts of the body. With the advent of the first complement therapeutics, and the probable arrival of more in the future, there seem to be multiple possible applications in the treatment of rheumatic diseases. The three rheumatic diseases highlighted in this review all display clear evidence of complement activation, also the animal models for these diseases indicate an active role for complement in the disease process. Strikingly at this moment therapeutic complement inhibition appears only to be effective in AAV and not in RA or SLE. Clearly there is a need for a more thorough understanding of the contribution of complement activation and complement regulation to the disease processes in rheumatic diseases.

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