Targeting complement in rheumatoid arthritis

Éva Biró¹, Paul P. Tak², Augueste Sturk¹, C. Erik Hack³, Rienk Nieuwland¹

¹Dept. of Clinical Chemistry, Academic Medical Center, University of Amsterdam
²Dept. of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam
³Crucell, Leiden
Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by polyarticular synovitis leading to cartilage, tendon and bone destruction, and pain and dysfunction of the joints. It is considered to be an immune-mediated inflammatory disorder, in which the complement system also plays a fundamental role. In the circulation of RA patients, increased levels of complement activation products have been found, often correlating with disease activity. In synovial fluid of the patients, decreased levels of native complement components and increased levels of activation products have been detected. Furthermore, in synovial tissue and cartilage, deposition of activated complement components has been demonstrated. As activators of the complement system in RA, immune complexes, C-reactive protein, and certain immunoglobulin G glycoforms have been identified. A role for complement activation in the pathogenesis of this disease is supported by studies showing an association between complement activation and inflammatory responses in the diseased joints or in individual cell types found in RA joints, and by extensive studies on animal models of the disease, utilizing for example animals deficient for certain complement components. Finally, several agents are under development to therapeutically influence the complement system, and some have already been tested in clinical trials of RA.
Rheumatoid arthritis (RA) is a chronic inflammatory disease with a complex pathogenesis, characterized by polyarticular synovitis leading to cartilage, tendon, and bone destruction, and pain and dysfunction of the joints [1,2]. Though the pathogenesis of RA is not clear, it is considered to be an immune-mediated inflammatory disorder, in which the complement system also plays an important role. This review, after giving a brief overview of the complement system, summarizes the data acquired to date supporting the pathogenetic role of the complement system in RA, and describes therapeutic advances aimed at influencing the complement system in this disease.

**Brief overview of the complement system**

**Activation pathways**

The complement system is a part of the innate immune system and plays a major role in the elimination of pathogenic microorganisms, the clearance of necrotic and apoptotic cells, and the processing of immune complexes. Depending on the activator, complement activation occurs either via the classical, the lectin, or the alternative pathway, as has been reviewed previously [3]. This can be a result of a direct interaction between the activating surfaces (target) and the complement components, or can be mediated by activator molecules that form complexes with the target.

The **classical pathway** can be activated either by immune complexes with immunoglobulin (Ig)M or IgG, by complexes with C-reactive protein (CRP) or serum amyloid P-component (SAP) [4-9], and also directly, by certain microorganisms. Upon binding of C1q to the target or to the activator molecule bound to the target, the two C1r serine proteases in the C1 complex (which consists of one molecule of C1q, two molecules of C1r, and two molecules of C1s) undergo autoactivation and then cleave and thereby activate the two C1s serine proteases, which subsequently cleave C4 and C2, forming the classical pathway C3 convertase (C4b2a). This, in turn, cleaves C3, and finally the classical pathway C5 convertase (C4b2a3b) is formed, see Figure 1.

The **lectin pathway** can be activated by terminal carbohydrate groups such as mannose on microorganisms, to which mannose-binding lectin (MBL) or one of the ficolins binds [10], after which a mannose-binding lectin-associated serine protease cleaves C4 and C2, similarly to the C1 complex, and again the classical pathway C3 convertase is formed, C3 is cleaved, and the classical pathway C5 convertase is formed (Figure 1).

The **alternative pathway** is activated directly by various microorganisms, and also by large complexes with IgG or IgA [11-13]. It begins with complement component C3, which is spontaneously and continuously activated at a low level (“tick-over” activation starting with spontaneous hydrolysis of an internal thioester bond in C3 by water). In the presence of an activator of the alternative pathway, full-blown activation occurs, with factor D cleaving factor B, formation of the alternative pathway C3 convertase (C3bBb) stabilized.
Figure 1. The complement system and current therapeutic targets. The classical, lectin and alternative pathways of complement activation are depicted. Dotted arrows indicate enzymatic cleavage. (Complement components C4 and C3 both contain an internal thioester bond, which becomes unstable after they’re cleaved, so that the cleavage fragments C4b and C3b attach covalently to hydroxyl and amino groups on the surface on which they were formed [201,202].) Inhibitors are shown within gray oval forms. Current specific therapeutic targets.

CR1, complement receptor-1; DAF, decay accelerating factor; MAC, membrane attack complex; MASP, mannose-binding lectin-associated serine protease; MBL, mannose-binding lectin; MCP, membrane cofactor protein.
by properdin, and finally the formation of the alternative pathway C5 convertase (C3bBb3b). The alternative pathway also becomes activated upon initiation of complement activation via the classical or the lectin pathways, and results in amplification of the response (Figure 1).

All three activation pathways lead to the activation of a common terminal pathway, where cleavage of C5 and subsequent association to C5b of C6, C7, C8 and several molecules of C9 lead to the formation of the membrane attack complex (MAC). This complex forms lytic pores in cell membranes (Figure 1).

**Effector mechanisms**

The activated complement system exerts its effects by various mechanisms [3]. During activation, small peptides, the anaphylatoxins C4a, C3a and C5a, are released, which interact with specific receptors on leukocytes causing chemotaxis and activation of these cells (with C5a being the most potent anaphylatoxin). C3b and C4b, as well as degradation fragments of these complement activation products act as opsonins, which when bound covalently to the target stimulate phagocytosis through binding to complement receptors (CR1 to CR4) on phagocytic leukocytes. Erythrocytes also possess one of these receptors, CR1, on their surface, and play a major role in the clearance of opsonized microorganisms and immune complexes by mediating transport to the mononuclear phagocytic system in the spleen and liver. CR1 (CD35) and CR2 (CD21) also play a role in promoting the antigen-specific activation of B cells. Finally, the MAC causes lysis of target cells by insertion into their membranes.

**Complement regulators**

Complement activation is regulated by a number of soluble and membrane-bound proteins that inhibit activation in the fluid phase in the absence of a target, and inhibit activation on cell surfaces of the host.

In the fluid phase, C1-inhibitor blocks C1r, C1s and the mannose-binding lectin-associated serine proteases [14,15]. Factor I, together with C4-binding protein serving as a cofactor, degrades C4b, and with factor H or factor H-like protein-1 [16] serving as a cofactor, it catabolizes C3b [17-19]. C4-binding protein and factor H (as well as factor H-like protein-1 [20]) also promote the dissociation of the classical and alternative pathway C3 convertases, respectively [18,19]. S-protein (vitronectin) and clusterin inhibit the formation of the MAC [21-24]. Furthermore, the anaphylatoxins are inactivated by plasma carboxypeptidases N and R (the latter also called carboxypeptidase U, plasma carboxypeptidase B, or thrombin activatable fibrinolysis inhibitor) [25,26].

On host cell surfaces, membrane cofactor protein (MCP; CD46) and CR1 act as cofactors to factor I, and decay accelerating factor (DAF; CD55) and CR1 promote the dissociation of the classical and the alternative pathway convertases [27-29]. Furthermore, CD59 (protectin) in host membranes inhibits the formation of the MAC [30,31].
In spite of this elaborate system for inhibition of inappropriate complement activation, the complement system can also have harmful effects besides its essential role in host defense and clearance of necrotic and apoptotic cells and immune complexes. It can for example contribute to the cardiovascular collapse when it is activated systemically on a large scale as in sepsis [32], or it can be a major cause of tissue damage when it is activated by necrosis of tissues that do not proliferate easily such as the myocardium [33-35], or when it is activated by autoimmune processes such as rheumatoid arthritis, as detailed below.

Role of the complement system in the pathogenesis of rheumatoid arthritis

Complement in the circulation and diseased joints of patients with rheumatoid arthritis

Initial reports on the complement system in RA date back to over 50 years ago. In the systemic circulation of the patients, total complement hemolytic activity (CH50; assessing activity of components of the classical and common terminal pathway) was reported to be normal or elevated [36-38], and in some (relatively severe) cases, decreased [39,40]. This is consistent with many complement proteins being positive acute phase reactants, with increased synthesis counteracting the consumption of these proteins upon complement activation [41,42]. Increased MBL levels have also been described in RA serum [43]. Studies analyzing activation products of complement in plasma or serum of RA patients showed increased levels of C1/C1-inhibitor complexes [44-47], sometimes correlating with joint inflammatory activity and sometimes not [44,47], and increased levels of C3 and C4 activation products [45,48-54], including the anaphylatoxins C3a [55], though at least one study found C3 activation products merely in 1/3 of the patients [56]. Several reports showed an association between C3 and C4 activation products and disease activity [48,49,51,53,55]. A metabolic turnover study of C3 in the circulation of RA patients found hypercatabolism of C3 mainly in patients with extra-articular manifestations of RA. Interestingly, neither levels of total C3 nor C3d, an activation product, correlated with C3 turnover, and decreased C3 levels were not found in any of the patients with hypercatabolism, indicating compensation by increased synthesis [57]. In a recent study, C1q-C4 complexes were measured in plasma of RA patients as novel, specific, and stable markers of classical pathway activation, minimally susceptible to in vitro artefacts [58,59]. These complexes were found at significantly higher levels in patients with active versus inactive RA, and correlated with disease activity. C4 activation products measured in these patients also correlated with C1q-C4 complexes [59]. Factor B fragments were also shown to be elevated in RA plasma [60], and an increased rate of catabolism of factor B, similarly
to that of C3, has also been associated with extra-articular manifestations [61]. A subsequent metabolic turnover study showed a higher catabolism of C4 than of factor B [62]. Levels of the fluid phase MAC were also elevated in RA plasma, though in one study again in only 1/3 of the patients [45,47,56,63]. Furthermore, elevated levels of the anaphylatoxin C5a were found [64]. Thus, although levels of native complement components in the circulation of RA patients may be normal or even elevated as a consequence of the acute phase reaction, levels of various activation products are increased, indicating activation of the complement system in these patients.

Complement factors in synovial fluid and synovial tissue are derived not only from plasma, but also from local synthesis of these proteins [65-68]. In synovial fluid of RA patients, complement hemolytic activity (CH50), levels of the classical pathway components C1, C4, and C2, as well as levels of complement component C3 were shown to be decreased [69-74], just as levels of the alternative pathway components factor B and properdin [75,76], compared with synovial fluid of patients with non-rheumatoid forms of arthritis. At the same time, activation products of C4, factor B, C3, and C5, including the potent anaphylatoxins C5a and C3a [50,55,56,64,75,77-83], as well as C1/C1-inhibitor complexes [46,47,83,84] and fluid phase MAC [47,56,81,82,85] were shown to be elevated in RA synovial fluid. These data indicate a strong activation of the complement system in the inflamed joints of RA patients. To what extent the circulating complement activation products are derived from the joints, or whether they originate from activation processes outside the joints, remains unclear.

Furthermore, in synovial tissue and cartilage of the diseased joints of RA patients, deposition of activated complement components was demonstrated, including that of C4 and C3 [86-89], the MAC [63,88-90], and active C1s [91]. Regarding the presence of complement regulators in synovial tissue, contradicting reports have been published. Reduced expression of DAF and absence of protectin have been described in synovial lining cells of RA patients [89,92], but this was not confirmed in other studies [67,93,94].

Regarding the cause of complement activation in RA, there is extensive evidence advocating a role for immune complexes, but other molecules such as CRP and certain IgG glycoforms have also been implicated. Immune complexes in synovial fluid were associated with increased consumption, i.e. activation of complement measured as a decrease in CH50 or native complement factors, or as an increase in activation products [82,95-97]. Colocalization of immunoglobulins with C3 was also shown in hyaline cartilage of RA patients [87]. Immune complexes or rheumatoid factor in the circulation of RA patients also correlated with complement activation products or metabolic turnover of complement [48-50,62]. Rheumatoid factors from serum or synovial fluid of RA patients or derived from Epstein-Barr virus-transformed B cells of RA patients were capable of activating complement in vitro as well [98-101]. However, a major role for circulating immune complexes in activating complement in RA has also been questioned. Levels of C1/C1-inhibitor complexes in plasma of RA patients, reflecting C1 activation in vivo, were
shown not to correlate with levels of circulating immune complexes, with levels of C1/C1-inhibitor complexes being only slightly increased in a part of the patients, whereas levels of circulating immune complexes were elevated in most of them. The activation of C1 by immune complexes of these patients in vitro was in line with the ex vivo results: C1 activation was (slightly) above normal in only 1/3 of the cases [102]. Similar discrepancies between measured immune complexes and C1/C1-inhibitor levels in serum were noted also in another study [44]. The role of another molecule, CRP, has been proposed in at least part of the activation of complement in RA. Complexes between CRP and activation products of C3 and C4 were shown to be specific for CRP-mediated classical pathway activation in vivo [103], and these complexes were elevated in the plasma of the majority of the patients with RA and also correlated with disease activity [54]. In a recent study, we found low levels of cell-derived microparticles with bound C1q, C4 and C3 on their surface in plasma of RA patients, indicating low-level classical pathway activation on the surface of these microparticles in the circulation of the patients. The levels of microparticles with bound C1q correlated with the levels of those with bound CRP, suggesting the role for bound CRP in the complement activation [104]. In synovial fluid of the patients, microparticles with bound C1q, C4 and C3 on their surface were present at very high levels, and correlation analyses indicated a role for IgG and IgM molecules bound to the microparticle surface in the classical pathway activation [104]. Finally, it has been shown that the extent of galactosylation of circulating IgG molecules in RA patients is decreased [105], and that these glycoforms lacking galactose can bind MBL and activate the lectin pathway of complement [106].

Pathogenetic consequences of complement activation in RA

That complement activation plays a role in the development of RA has been suggested by several studies showing an association between complement activation and inflammatory responses in the diseased joints of RA patients or in individual cell types found in synovial tissue. C3 activation products correlated with synovial fluid leukocyte counts and with lactoferrin, a parameter of neutrophil activation [50,83,107]. Furthermore, non-lethal MAC incorporation into membranes of RA synovial cells in culture stimulated the release of reactive oxygen metabolites, prostaglandin E2, leukotriene B4, and interleukin (IL)-6 from these cells [108-110], and C5a stimulation of synovial mast cells from RA patients induced the release of substantial amounts of histamine from these cells, which also showed increased expression of the C5a receptor compared to synovial mast cells from patients with osteoarthritis [111]. C3 degradation products also correlated with the clinically assessed level of joint inflammation in RA patients [112].

More direct evidence demonstrating the pathogenic role of the complement system in RA came from studies performed using animal models of the disease. In murine models of RA, deficiency of C1q, C4, a combined deficiency of CR1 and CR2, or a deficiency of CR3 had no effect [113,114], while deficiency of factor B and especially of complement
component C3 ameliorated the disease [114-116], suggesting a predominant role of the alternative pathway in the development of arthritis. Deficiency of the C3αR did not protect against arthritis in a mouse model [117]. Deficiency of C6 also had no effect in one study, suggesting that the MAC was also not required [114]. This was, however, contradicted by another study using a rat model of arthritis, showing that deficiency of C6 effectively reduced disease severity [118]. Mice deficient for C5 were resistant to developing arthritis [114,119-122], as were mice deficient for the C5α receptor (C5αR) [114,117], suggesting a central role for this complement component in the pathogenesis of RA, though this could not be confirmed in all studies [123,124], perhaps due to concomitant genetic variations in the C5-deficient mice. Further evidence implicating C5 involves its interaction with Fcγ receptors (FcγR). Several studies have indicated an activating role in mediating joint inflammation for FcγRIII and to a lesser extent FcγRI in mouse arthritis models, and an inhibitory role for FcγRII [125-127], and it was shown that C5α can increase expression of the activating FcγRIII and reduce the expression of the inhibitory FcγRII in a C5αR-dependent manner, thereby augmenting inflammatory responses in vitro and in vivo in a mouse model of acute pulmonary immune complex hypersensitivity [128]. In a serum transfer model of RA in mice (using serum from arthritic K/B×N mice), the expression of FcγR as well as that of C5 and C5αR were necessary for recruitment and activation of inflammatory cells and the development of arthritis [114], presumably via a mechanism similar to the one described in the pulmonary hypersensitivity model [129].

The possible role of complement regulatory proteins in the development of RA has also been studied using animal models. In a rat model of the disease, the inflammation was further increased by blocking the membrane complement regulators Crry (a rodent functional homologue of human MCP and DAF [130,131]) and CD59, using F(ab’)2 fragments of monoclonal antibodies against these two proteins injected intra-articularly [132]. In line with this, the arthritis in mice transgenic for Crry was suppressed [133]. Furthermore, the arthritis in a murine model was worsened if the animals were also deficient for CD59a (a form of mouse CD59 that is widely distributed in tissues [134]), the effect of which was reversed by intra-articular administration of membrane-targeted soluble CD59 (sCD59-APT542) [135]. An effect of CD59 inhibition or deficiency also suggests a role for the MAC in the development of arthritis in these experiments, what contradicts results of other studies. The discrepancies are probably caused by differences between the animal models used, and it still awaits clarification whether C5α or the MAC, or both are important in human RA.

Further extensive evidence implicating the complement system in the pathogenesis of RA came from studies testing the effects of complement inhibitors as potential therapeutics in animal models or clinical trials of RA.
 CHAPTER 8

Therapeutic inhibition of the complement system in rheumatoid arthritis

Several (potential) therapeutic agents, such as neutralizing antibodies or antibody fragments, synthetic antagonists, engineered soluble forms of natural complement inhibitors, or synthetic protease inhibitors target the complement system directly (see Figure 1 for current specific therapeutic targets within the complement system). Beside these, other therapeutics that have a primary target other than a component of the complement system, such as anti-cytokine therapy, or lipid-lowering therapy with statins, can also have consequences for complement activation.

Direct inhibition of the complement system

Anti-C5 antibodies

Studies on C5- and C5aR-deficient animals (see above) have suggested an important role for C5 in the development of arthritis. In accordance with those results, systemic administration of a monoclonal antibody against murine C5, blocking the generation of C5a and the formation of the MAC, effectively prevented the onset of arthritis and ameliorated established disease in a murine model of RA [136,137]. The same results were obtained with anti-C5 in another model of murine arthritis [114]. Administered intra-articularly, an anti-C5 single-chain fragment variable antibody (scFv), TS-A12/22, which also inhibits the cleavage of C5, thereby inhibiting both C5a release and formation of the MAC, and another scFv antibody (TS-A8) that selectively blocks the MAC formation, were both effective in a rat model of arthritis [118,138].

Eculizumab (h5G1.1; Alexion Pharmaceuticals, Inc., Cheshire, CT, USA) is a humanized monoclonal antibody that inhibits the cleavage of C5 by the classical and the alternative pathway C5 convertases, and thereby inhibits the formation of both C5a and the MAC [139]. It has proven to be an effective treatment for patients with paroxysmal nocturnal hemoglobinuria, blocking complement activation, reducing intravascular hemolysis and increasing the quality of life, with no significant adverse effects [140,141]. Eculizumab has also been tested in patients with RA, but did not meet expectations regarding clinical efficacy [142].

Pexelizumab (h5G1.1-scFv; Alexion Pharmaceuticals, Inc., Cheshire, CT, USA) is a recombinant, humanized, monoclonal, single-chain antibody fragment version of the antibody 5G1.1, with the same effects on the complement system as eculizumab [139]. To date it has been tested in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB), and in patients with acute myocardial infarction (AMI). It proved to be a safe and effective inhibitor of pathological complement activation in patients undergoing cardiac surgery with CPB, reducing soluble MAC formation, leukocyte activation, postoperative myocardial injury, cognitive deficits, and blood loss [143-149]. In a phase II study on
patients with AMI undergoing primary percutaneous coronary intervention (PCI), pexelizumab had no measurable effect on infarct size, but reduced mortality significantly [150]. This effect was associated with decreased CRP and IL-6 levels in these patients, suggesting that the clinical benefit was mediated through anti-inflammatory effects [151]. In contrast to these results, in AMI patients treated with thrombolitics, pexelizumab had no effect [152], nor in a phase III study on AMI patients undergoing primary PCI [153], questioning the effectiveness of this therapy in AMI. Pexelizumab has not been tested in RA.

**C5aR antagonists**

Intra-articular or intravenous injection of the synthetic hexapeptide C5aR antagonist NMePhe-Lys-Pro-dCha-Trp-dArg was ineffective in a rat model [154,155]. In contrast, an orally active C5aR antagonist, the cyclic peptide AcF-[OPdChaWR] (PMX-53) significantly reduced joint pathology in another rat model [156]. This latter compound has also been tested in RA patients in a phase Ib clinical trial, where it did not result in a reduction of synovial inflammation [157].

**C3aR antagonists**

SB 290157, a nonpeptide antagonist of the C3aR, has been shown to reduce inflammation in a rat arthritis model [158]. However, this peptide was later shown to possess agonist activity as well in a variety of systems [159].

**sCR1**

Intra-articular administration of soluble CR1 (sCR1) reduced the development of arthritis in a rat model, but was ineffective when administered systemically [155,160]. Trials of recombinant sCR1 TP-10 (AVANT Immunotherapeutics, Needham, MA, USA) in patients with acute lung injury or acute respiratory distress syndrome and in adult and pediatric patients undergoing cardiac surgery with CPB showed that it was well tolerated, significantly decreased mortality and AMI in male patients undergoing cardiac surgery, and appeared to decrease complement activation in infants undergoing cardiac surgery [161-163].

A membrane-targeted form of human sCR1, APT070, targeted using a synthetic address tag comprised of a lipid moiety that interacts with the hydrophobic interior of the plasma membrane and a short positively charged peptide that interacts with negatively charged phospholipid head groups (APT542) [164], reduced the development of arthritis in a rat model when injected intra-articularly [165].

Gene therapy with sCR1 has also been tested in a murine model of arthritis, by injection of retrovirally infected syngeneic cells expressing truncated sCR1, or by injection of naked DNA containing truncated sCR1 and dimeric truncated sCR1 coupled to an IgG
heavy chain fragment (truncated sCR1-Ig). Both forms of therapy inhibited the development of arthritis in the mice [166].

**DAF-Ig**
Coupling recombinant soluble forms of DAF or CD59 (or both) to IgG Fc fragments generated molecules with an extended half-life *in vivo*, though with significantly decreased activity compared to the soluble non-fusion protein variants. Nevertheless, intra-articular administration of DAF-Ig significantly reduced the severity of arthritis in a rat model [167].

**Membrane-targeted soluble CD59**
Intra-articular administration of membrane-targeted soluble CD59 (sCD59-APT542), targeted using the same synthetic address tag (APT542) as the sCR1 derivative APT070 [164], suppressed arthritis in a rat model [168], and reversed the effect of CD59 deficiency in a murine arthritis model [135].

**Nafamostat mesilate (FUT-175)**
Nafamostat mesilate or FUT-175 is a synthetic protease inhibitor also blocking proteases of the classical as well as the alternative pathway of complement activation [169,170]. Administered orally, it inhibited arthritis in a rat model [171]. This agent is already used in patients, for example in the treatment of acute pancreatitis, patients undergoing cardiac surgery with CPB, or for anticoagulation during hemodialysis [172-174].

**Indirect inhibition of the complement system**

**Infliximab**
CRP-mediated classical pathway activation has been shown to be elevated in RA patients suggesting that at least part of the complement activation occurring in this disease is mediated by CRP [54]. Treatment of RA patients with infliximab, a chimeric monoclonal antibody against tumor necrosis factor α (TNFα), decreases plasma levels of CRP [175,176], and it has also been shown to decrease CRP-dependent complement activation products [177]. Treatment with infliximab can thus indirectly reduce complement activation in RA patients.

**Statins**
On endothelial cells *in vitro*, statins have been shown to increase the expression of DAF, and under hypoxic conditions also the expression of CD59, which prevents complement activation on these cells [178,179]. It was suggested that increased expression of DAF and CD59 and inhibition of complement activation may contribute to the anti-inflammatory effects of statins in RA [179], since the rheumatoid joint is also known to be a hypoxic environment [180,181].
Statins are also known to decrease CRP levels [182,183], and may also in this way lower complement activation in RA patients, similarly to infliximab.

**General considerations regarding therapy targeting the complement system in RA**

Inhibition of the complement system can have unwanted side effects, such as increased susceptibility to infection and autoimmune disorders [3,184,185], and impaired repair processes in various organs [186-188]. These potentially harmful side effects can be minimized by carefully choosing the target of inhibition and by optimization of the dosing, whereby the complement system is suppressed sufficiently for a therapeutic effect but not completely inhibited. Agents preventing C3 activation such as sCR1 or DAF have more serious side effects compared with agents that act downstream of C3 in the complement cascade, such as anti-C5 antibodies, C5aR antagonists and CD59. Agents acting downstream of C3 activation still allow formation of C3b and thereby opsonization, phagocytosis, immune complex clearance, and enhancement of B cell responses. Local instead of systemic inhibition of the complement system, by local delivery of therapeutics or by targeting of these reagents, can also prevent unwanted systemic side effects, and increase efficacy. Examples of such reagents are APT070, a membrane-targeted form of sCR1, or sCD59-APT542 [135,164,165,168].

Further important points to consider when designing anti-complement therapeutics are cost and ease of manufacture, *in vivo* stability and half-life, and the potential to elicit antibody responses that would inhibit the therapeutic effect. Expression of complement regulators as Fc fusion proteins, for example, generates complement inhibitor reagents with extended half-lives *in vivo* [189], and production of humanized or human monoclonal antibodies can minimize the problems of antigenicity [139,190].

**Future perspectives**

Over the years, a tremendous amount of experimental data has been collected regarding the pathogenesis of rheumatoid arthritis and the role of the complement system therein. It is now certain that the complement system plays an important role, but the precise mechanism or mechanisms still need to be clarified. Several agents interfering with the complement system are effective in animal models, but regarding human RA, therapeutic attempts targeting the complement system have been less successful. Improvements in this respect might be expected from gathering more precise knowledge regarding the pathogenesis of the disease, and further innovations in drug design.

An area of investigations expected to make substantial contributions to our understanding of the development of RA in the coming years is for example the identification of the various genetic loci, which are associated with an increased risk of this
disease. Of potential interest regarding our knowledge of the role of the complement system in the pathogenesis of RA is the recent finding, obtained using genome-wide association analyses as well as a candidate gene approach, that a polymorphism in the TNF receptor-associated factor 1 (TRAF1)/C5 region on chromosome 9 is associated with an increased susceptibility to RA. Further studies will be needed to establish precisely which locus is involved and what biological processes are altered by the RA-associated genetic variant(s) [191,192].

Potential therapeutic agents not yet tested in arthritis models are for example a truncated version of sCR1, which lacks the C4b binding site and is a selective inhibitor of the alternative pathway [193], antisense peptides such as those that have been designed to inhibit C5a [194,195], or stabilized RNA called aptamers that bind C5 for example, and inhibit its cleavage by the classical or alternative pathway C5 convertases [196]. Also, DAF-Ig prodrugs (fusion proteins comprised of DAF and IgG Fc) with an extended half-life in vivo, but significantly decreased activity compared to the soluble non-fusion protein variants have been developed, with specific cleavage sites for matrix metalloproteases and/or aggrecanases [197]. These enzymes can both be found in the diseased RA joints [198,199], and by releasing soluble DAF, they restore its activity, which is thus localized to the area of inflammation. Genetic engineering of complement regulators to increase their inhibitory activity also seems feasible [200], and gene therapy might also be an option in the future [166].

References


TARGETING COMPLEMENT IN RHEUMATOID ARTHRITIS


selective nonpeptide antagonist of the anaphylatoxin C3a receptor that demonstrates antiinflammatory activity in animal models. *J Immunol* 2001; 166: 6341-6348.


CHAPTER 8