Complement and its breakdown products in SLE

G. Sturfelt and L. Truedsson

The complement system has important protective functions in both the innate and the adaptive immune systems but can also, when inappropriately activated, cause tissue damage. Complement deficiency predisposes to infection and also to development of autoimmune disease, especially SLE, and complement is at the same time involved in the pathogenesis of this disease. In this review, various aspects of this dualism are discussed. An overview of activation pathways and activation products is given, together with a description of autoimmunity against complement and the potential of complement regulation in future therapeutics.

KEY WORDS: Complement, SLE.

The complement system consists of a large number of proteins, some of which are soluble plasma constituents and others cell membrane proteins. A main task of complement components is to stop invasion by microbes; membrane complement proteins act as receptors for target-bound complement. Regulators of complement activation protect self tissue from damage [1]. During recent years it has become more and more obvious that the complement system is not only involved in killing of invasive pathogens, but also contains important regulators and activators of several humoral and cellular immune functions [2]. The complement system bridges innate and adaptive immune responses, and in this review we will discuss the dualistic role of complement as a mediator of protective functions and as a cause of tissue damage, with focus on the pathogenesis of systemic lupus erythematous (SLE).

Complement function: crossroads and dualism

The complement system exerts a number of important functions in defence against infection as a part of the innate immune system and as a mediator of effects elicited by the adaptive immune system [1]. To destroy targeted microbes, this is accomplished by creating chemotactic signals and opsonization, which supports phagocytosis and in some cases direct killing. Furthermore, regulatory effects of complement on B-cell function and on cytokine production by, for instance, dendritic cells and scavenging of necrotic and apoptotic cellular debris are important functions that have been discussed not only with regard to infections but also to autoimmune diseases, especially SLE [3].

The actions of complement require activation of one of three sequences or pathways, all generating C3 convertase: the classical pathway, the mannan-binding lectin (MBL) pathway and the alternative pathway (Fig. 1). Covalent binding of C3b to hydroxyl groups on carbohydrates and amino groups on proteins is enabled by activation of an internal thioester bond. The classical pathway is mainly initiated by interaction of C1q with antibodies [IgG and IgM] in immune complexes and is the main effector of antibody-mediated immunity. Generation of the classical pathway convertase C4b2a can also occur via the MBL pathway independently of C1 activation, by activation of the serum protease mannan-binding lectin-associated serine protease (MASP)-2. Activation of the alternative pathway occurs by spontaneous hydrolysis of the internal thioester bond in C3 followed by interaction with factors B and D. This creates the alternative pathway C3 convertase C3bBb, which is stabilized by properdin, the only positive regulator protein of the complement system. Covalent binding of cleaved C3, (ie C3b) to microorganisms or immune aggregates leads to activation of the terminal components with production of the C5a fragments and assembly of the membrane attack complex (C5b–9). During recent years it has been shown that the MBL pathway and the alternative pathway are capable of discriminating between pathogens and self according to the concept of pattern of recognition receptors [4].

The complement receptors constitute interfaces between activated complement components and various cell phenotypes. Several cell-associated C1q-binding proteins have been described, including C1qR and gC1qR, which bind the collagen-like region and globular heads of C1q, respectively [5]. CR1 (CD35) is a widely distributed receptor, binding C3b, iC3b and C4b, and probably also serves as a C1q receptor. CR2 (CD21) is a receptor for C3dg and is also used by Epstein–Barr virus. CR2 exhibits several activities. Ligation of CR2 with antigen-bound C3dg and binding of antigen to the B-cell receptor results in B-cell activation and proliferation. CR3 (CD11b/CD18) and CR4 (CD11c/CD18) are members of the integrin family and are important in mediating phagocytosis.

Self surfaces are protected from complement activation products by a series of proteins such as CR1 (CD35), CRIT, DAF (CD55), MCP (CD46) and protectin (CD59) [6, 7]. In the circulation, the control of complement activation at the C1 and MBL-MASP levels is accomplished by C1 inhibitor (C1INH). Inhibition at the C3 level is mediated by C4BP and factors I, H and HL-1 [8]. S-protein and clusterin inhibit incorporation of the membrane attack complex in membranes [9].

During activation, peptides and complexes are formed that have a wide variety of biological effects, including tissue damage and protective functions. An overview is given in Table 1.
Complement deficiency in man can be inherited or acquired. The latter is usually partial and due to complement breakdown that is sometimes associated with the occurrence of complement autoantibodies. Deficiencies within all pathways increase the risk of infections. In addition, classical pathway deficiencies are associated with the development of autoimmune disease, mainly SLE and SLE-like disorders (Table 2).

Deficiencies of C1q, C1r/C1s, C4 and C2 are associated with a high risk of pyogenic infections. In long-term follow up of 40 C2-deficient (C2D) individuals, 23 had suffered from severe invasive infections, mainly septicaemia or meningitis caused by Streptococcus pneumoniae, and 19 had at least one episode of pneumonia; recurrent pneumonia was documented in 10 patients. Repeated infections occurred mainly during infancy and childhood [10].

MBL deficiency probably contributes to susceptibility to infection. Thus, MBL deficiency has been reported in repeated respiratory infections and may be associated with poor prognosis in cystic fibrosis [11, 12]. Furthermore, MBL deficiency has been associated with a high frequency of pulmonary infections in SLE [13].

Deficiency states within the classical pathway are associated with increased risk of developing autoimmune rheumatological disorders, especially SLE and SLE-like disease. A clear hierarchy exists, according to which deficiency of C1q (C1qD) and C1r/C1s is associated with a high risk of developing clinical disease, while

---

**TABLE 1A. Complement split products and complexes formed during activation and their biological functions: split products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Molecular weight (kDa)</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2a</td>
<td>68</td>
<td>Larger fragment when C2 is cleaved during activation. Part of the C3 convertase C4b2a&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2b</td>
<td>30</td>
<td>Smaller fragment when C2 is cleaved during activation</td>
</tr>
<tr>
<td>C4a</td>
<td>8</td>
<td>Smaller fragment when C4 is cleaved during activation. Complement anaphylatoxin</td>
</tr>
<tr>
<td>C4b</td>
<td>192</td>
<td>Larger fragment when C2 is cleaved during activation. Part of the C3 convertase C4b2a. Binds to CR1</td>
</tr>
<tr>
<td>C3a</td>
<td>8</td>
<td>Smaller fragment when C3 is cleaved during activation. Complement anaphylatoxin. Chemotactic for eosinophil granulocytes. Direct anti-microbial effect [49]</td>
</tr>
<tr>
<td>C3a-desArg</td>
<td>8</td>
<td>Inactivated derivative of C3a. Chemotactic to eosinophil granulocytes. Direct anti-microbial effect [49]</td>
</tr>
<tr>
<td>C3b</td>
<td>178</td>
<td>Larger fragment when C3 is cleaved during activation. Part of the C3 convertase C3bBb&lt;sup&gt;b&lt;/sup&gt; and the C5 convertases C4b2a3b and C3bnBb. Binds to CR1 (opsonization, regulation of activation)</td>
</tr>
<tr>
<td>iC3b</td>
<td>176</td>
<td>Inactivated C3b. Binds to CR3, CR4 (opsonization) and weakly to CR1</td>
</tr>
<tr>
<td>C3c</td>
<td>138</td>
<td>Degradation fragment formed by cleavage of C3b</td>
</tr>
<tr>
<td>C3d/dg</td>
<td>33–35/38</td>
<td>Degradation fragments of C3b. May be covalently bound to a target molecule. Binds to CR2 on B cells. Physiological adjuvant of antibody responses [50]</td>
</tr>
<tr>
<td>C5a</td>
<td>11</td>
<td>Smaller fragment when C5 is cleaved during activation. Complement anaphylatoxin. Chemotactic for neutrophil granulocytes and monocytes. Induction of apoptosis [51] and delay of apoptosis [52]</td>
</tr>
<tr>
<td>C5a-desArg</td>
<td>11</td>
<td>Inactivated derivative of C5a. Chemotactic for neutrophil granulocytes and monocytes</td>
</tr>
<tr>
<td>C5b</td>
<td>185</td>
<td>Larger fragment when C5 is cleaved during activation. Part of the membrane attack complex, C5b-C9a</td>
</tr>
<tr>
<td>Ba</td>
<td>30</td>
<td>Smaller fragment when factor B is cleaved during activation</td>
</tr>
<tr>
<td>Bb</td>
<td>63</td>
<td>Larger fragment when factor B is cleaved during activation. Part of the AP C3 convertase C3bBb and C5 convertase C3bBb. Induction of apoptosis [55]</td>
</tr>
</tbody>
</table>

---

**TABLE 1B. Complement split products and complexes formed during activation and their biological functions: complexes**

<table>
<thead>
<tr>
<th>Complex</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1INH-C1r-C1s</td>
<td>Intermediate complex formed during activation of C1</td>
</tr>
<tr>
<td>C1INH&lt;sub&gt;a&lt;/sub&gt;-C1r-C1s</td>
<td>Complex formed during activation of C1</td>
</tr>
<tr>
<td>C4b2a</td>
<td>CP C3 convertase</td>
</tr>
<tr>
<td>C4b2a3b</td>
<td>CP C5 convertase</td>
</tr>
<tr>
<td>C3bBb</td>
<td>AP C3 convertase</td>
</tr>
<tr>
<td>C3b&lt;sub&gt;b&lt;/sub&gt;Bb</td>
<td>AP C5 convertase</td>
</tr>
<tr>
<td>C5b-7, C5b-8</td>
<td>Precursors of the membrane attack complex. May be bactericidal for Gram-negative bacteria [54]</td>
</tr>
<tr>
<td>C5b-9&lt;sub&gt;n&lt;/sub&gt;</td>
<td>Membrane attack complex. Induction of cell proliferation [55] and apoptosis [56]</td>
</tr>
<tr>
<td>SC5b-9&lt;sub&gt;n&lt;/sub&gt;</td>
<td>Soluble non-lytic complex containing S-protein, also called soluble terminal complement complex (TCC)</td>
</tr>
</tbody>
</table>

---

<sup>a</sup>Classical and MBL pathway; <sup>b</sup>alternative pathway.

---

**Complement deficiency**

Complement deficiency in man can be inherited or acquired. The latter is usually partial and due to complement breakdown that
The paradox was that deficiency states within the classical pathway did not protect from SLE but strongly predisposed to the disease.

It has become more and more obvious that the pathogenesis of SLE is complex, and is initiated and modified by multiple genes and environmental factors. The evidence that products of complement activation really contribute to tissue damage in SLE is somewhat circumstantial, even if the phlogistic effects of complement are well documented. The support for pathogenetic effects of complement is based primarily on histopathological correlations of immune complex and complement deposits with clinical findings, studies on eluates from affected tissues, such as kidney, correlations between circulating, deposited and eluted immune complexes, and from animal studies.

Genes involved in scavenger mechanisms, tolerance and cytokine production have been suggested to be involved in SLE based on findings in humans and murine models. Thus, polymorphisms of interferon regulatory genes [18] in human SLE and of CRP/SAP, DNASE1, C1q, Fcγ receptors and PDCD1 in human SLE and murine models of SLE have been demonstrated to be important for susceptibility of the disease [19, 20].

Genetically determined deficiency of CR1 and CR2 is associated with severe SLE in animal models. In humans with SLE, CR1 deficiency is a mainly acquired phenomenon and not a primary genetic defect [21].

Several concepts concerning pathogenetic mechanisms, partly based on the relation between complement deficiency and SLE, have been put forward. One of the first hypotheses was that impaired handling of immune complexes was a major pathogenetic factor in SLE and such disturbances were also demonstrated in a large number of studies. This idea is complementary to the so-called waste disposal hypothesis [22]. Apoptotic cells are considered to be the main source of autoantigen in SLE, and C1q, together with some other proteins, such as C-reactive protein (CRP) and natural IgM, are recognition molecules. Some studies have indicated, but not definitely demonstrated, that these models explain the hierarchical relationship between C deficiency and the risk of developing SLE. According to another hypothesis, the complement system protects against immune response to autoantigens by enhancing the elimination of self-reactive lymphocytes. Thus, complement-coated self antigens are delivered to developing B cells binding to CR1 (CD35) and CR2 (CD21) and enhance their negative selection. Complement deficiency would then result in lack of normal B-cell tolerance [23]. Another possibility is that complement components are important for the regulation of the cytokine production involved in SLE pathogenesis, for instance by dendritic cells [24]. Examples of such cytokines are type 1 interferons, which in a series of studies have been shown to have a central role in the pathogenesis of SLE [25]. The possibility that complement deficiency can lead to persistent viral infections, which could be of importance in initiating an SLE process, has also been discussed. Figure 2 shows a diagram of proposed hypotheses on how complement dysfunction may influence the pathogenesis of SLE.

To protect from complement-induced damage of self tissues, host cells express a number of regulatory proteins. However, leucocytes can be activated through deposition of membrane attack complex (C5b-9) and the binding of C4b and C3b to complement receptors on leucocytes, leading to cell activation. Furthermore, as can be seen from Table 1, C5a and partly also C3a have chemotactic and anaphylactic effects. Deposition of C5b-9 in the kidneys and lungs associated with release of prostaglandins and pro-inflammatory cytokines has been reported and related to tissue damage [26]. In the kidneys, tubular cells lack regulatory proteins on the luminal side which implies that complement may cause tubulointerstitial damage [27]. However, the pathogenetic mechanisms in SLE are complex
Autoantibodies to complement components

Complement components may be targets of autoantibody responses. Autoantibodies may occur against individual components, convertase complexes, complement-regulating proteins and complement receptors. It has been demonstrated that autoantibodies usually bind to modified proteins or neo-antigens and are not reactive with native components. The presence of autoantibodies may be, but is not always, associated with disease manifestations.

In patients with SLE, autoantibodies against components of the classical pathway are often found. Immunoconglutinins recognize particle-bound C3 and C4 fragments and may interfere with factor I-mediated cleavage of C3 and thereby have regulatory effects on immune complex clearance. Anti-C1q autoantibodies are seen in virtually all patients with hypocomplementaemic urticaria vasculitis syndrome (HUVS) and approximately 30–50% of SLE patients. Most SLE patients with anti-C1q autoantibodies have glomerulonephritis of WHO class III–IV. Approximately 30% of HUVS patients develop nephritis (A. G. Sjöholm, U. Mårtensson, L. Truedsson and G. Sturfelt, unpublished observations) [29]. These antibodies have been suggested to be involved in the pathogenesis of SLE nephritis by binding to C1q planted on antibodies, or other C1q binding to structures in the glomeruli [30]. C1q-specific IgG recognizes the collagenous fragments of C1q molecules in bound form. In HUVS, C1q antibodies usually bind to the B and C chains of C1q [31].

Autoantibodies against C1s have also been reported in SLE but a pathogenetic effect of C1s autoantibodies has not been defined. C1INH is the inhibitor of C1 and of MASPs and is thus important for control of C4b2a generation. C1INH deficiency is usually caused by a genetic defect causing hereditary angio-oedema or by increased catabolism leading to acquired angio-oedema. Many patients with acquired angio-oedema have autoantibodies against C1INH and such autoantibodies can also occasionally be found in patients with SLE. C3 and C4 nephritic factors are IgG autoantibodies that stabilize the alternative pathway C3 convertase and the classical pathway C3 convertase, respectively. Both these autoantibodies can be found in SLE and have been associated with poor prognosis regarding renal involvement [32]. Autoantibodies against other complement regulators, such as CR1, CR3 and C1q receptor have also been reported in SLE and may be of pathogenetic importance by affecting the clearance of immune complexes and lupus antigens such as apoptotic material.

Complement as a biomarker of SLE in diagnosis and during follow-up

Observation of low complement concentrations and also of activation of the complement system are characteristic findings in active SLE and have led to the practice of using measurement of complement for the diagnosis, classification and monitoring of disease course in SLE.

In the 1982 set of ACR criteria for SLE, complement components were not included. However, low levels of CH50, C3 and C4 were tested when the criteria were developed. The sensitivities and specificities for the tested components were 70 and 70%, respectively, for CH50, 64 and 91% for C3 and 64 and 65% for C4. In further analysis by recursive partitioning on the same data set, the combined sensitivity and specificity for CH50, C3 and C4 were 74 and 88%. In a more recent study, low levels of C1q were found to have a high specificity for SLE (96%) but the sensitivity was low (20%) [32]. The observation of low CR1 and deposition of C4 and C3 fragments on erythrocytes in active SLE was made by Walport and colleagues [33, 34]. Recently, Manzi et al. [35] demonstrated that the combined detection of high levels of erythrocyte-bound C4d and low levels of CR1 on erythrocytes had high sensitivity (72%) and specificity (79%) for SLE. The possibility of interference by anti-CR1 autoantibodies and immunoconglutinins with the assay has not been evaluated.

Low concentrations of complement components due to increased catabolism are found in a majority of patients with active and severe SLE [36–38]. It was demonstrated by several groups that low C1q levels were associated with proliferative glomerulonephritis (WHO class III and IV) and that C1q concentrations decreased prior to clinical manifestations of flares of the disease [39–41]. Low C1q levels have also been shown to predict the histopathological outcome of lupus nephritis [41]. Autoantibodies against the collagen-like region of the C1q molecule are often but not always found in these cases. Disappearance or decreased concentrations of these antibodies associated with normalization of C1q is an important
favourable prognostic sign. It is important to realize that sequential measurements are required for the use of complement in monitoring disease activity in SLE.

In vivo complement activation can be demonstrated by tests for complexes or split products formed during complement activation, shown in Table 1. In a number of studies, such measurements have been shown to be more sensitive than regular measurement of CH50 or complement components such as C1q, C4 and C3 [40, 42–44]. Measurement of C2a, C3a, C3d, C4d, Bb and Ba in plasma have been reported to correlate with disease activity and predict flare of the disease. In several reports, C3d measurement in urine has been suggested to be of value for evaluation of active SLE glomerulonephritis [43]. In sequentially followed patients, evidence of activation of the early part of the classical pathway measured by C1INH2–C1r–C1s complexes is detected prior to C3 activation [42]. Since complement activation occurs in several comorbidities of SLE, especially infections, the specificity is low, even if the assays well discriminate active disease from remission of the disease. Furthermore, artefacts caused by complement activation in vitro during coagulation and other handling procedures are a problem. However, with proper handling of EDTA (ethylene diamine tetraacetic acid), plasma and addition of protease inhibitors, such assays might be of clinical value, since they reflect variations in complement-mediated handling of immune complexes and apoptotic material.

Treatment with complement and complement inhibition

Complement is involved in pathogenesis and contributes to organ damage in multiple diseases, including immune complex disorders and complement activation induced by ischaemia. During recent years pharmacological control of complement activation has been examined in various conditions with the purpose to reduce tissue damage [45]. Anti-complement treatment with monoclonal anti-C5 antibodies and C1INH has been demonstrated to reduce tissue damage in myocardial infarction and stroke [46]. In hereditary angio-oedema, C1INH effectively stops attacks of oedema and is also useful for prophylaxis. Anti-C5 antibodies have been proposed for trials in chronic inflammation in, for instance, rheumatoid arthritis, nephritis and dermatomyositis [47].

Membrane-associated regulators of complement activation have been suggested as potential therapeutic agents. Extracellular portions of these proteins may circulate as soluble proteins, often retaining complement-regulating function. Recombinant soluble CR1 (sCR1) inhibits formation of C3 and C5 convertases, C5a generation and MAC formation, and has been shown to inhibit cardiac damage in murine models of myocardial infarction [48]. However, the experience from trials in man has been to some extent disappointing and sCR1 is unlikely to become a major therapeutic alternative in the future.

Antibacterial effects of C3d fragments, which is due to binding to the complement receptor CR2 on B cells, have been utilized in vaccine development. Increased antibody responses have been achieved by conjugating various antigens to C3d. Recently this has been shown for DNA vaccines expressing soluble CD4 envelope proteins, which could elicit cross-reactive neutralizing antibodies to HIV-1 [50].

The adjuvant effect of C3d fragments, which is due to binding to the complement receptor CR2 on B cells, have been utilized in vaccine development. Increased antibody responses have been achieved by conjugating various antigens to C3d. Recently this was shown for DNA vaccines expressing soluble CD4 envelope proteins, which could elicit cross-reactive neutralizing antibodies to HIV-1 [50].

Complement therapies may in the near future become adequate alternatives for short-term complement inhibition in acute clinical situations. Long-term treatment can be anticipated to be complicated by an increased risk of infections and also autoimmunity disease. Future developments may, however, provide a basis for selective and effective regulation of complement activation and thereby open pathways towards therapeutic control of complement in disease.

Acknowledgement

We would like to express our gratitude to our good friend and mentor A. G. Sjöholm for careful reading of the manuscript.

The authors have declared no conflicts of interest.

References