The double-edged sword of complement activation during sepsis

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Introduction. Sepsis and septic shock are major killers world-wide. Antibiotic treatment and early-goal directed therapy in the intensive care unit are essential elements of care, but no adjunctive immune-modulatory treatment to alter the outcome has proven to be effective. The complement system plays a protective role in infectious diseases acting locally to kill the microbe, but during sepsis, complement may be activated systemically to induce an undesired excessive inflammation with subsequent tissue damage. This review discusses the diverse actions of the complement system in sepsis, emphasising on the defence against infection and the damage done by uncontrolled systemic activation of the complement system.

The complement system

Our first line of defence against infections is formed by the innate immune system. The innate immune system comprises a plasmatic part in which the complement system is most important, and a cellular part that recognises microbes using receptors such as the toll-like receptors (TLRs). Because components of the complement system circulate in the plasma as inactive enzymes that are ready to jump into gear upon activation, it is the only defence against infections that can be instantaneously activated when a pathogen enters the body. Thus, it is crucially important in fulminating infections since the other defence systems take more time to start up. The complement system in infectious diseases is a redundant system with diverse functions: 1. recognition of pathogens 2. elimination of these pathogens 3. signalling danger 4. removal and repair of apoptotic immune cells and damaged tissue, and 5. prevention of excessive activation.

Recognition of pathogens by the complement system is done by the initial pathways for complement activation: the classical, lectin and alternative pathways. Both the classical and lectin pathways use specific proteins designed to recognize pathogens for this aim. Classical pathway activation requires binding of antibody (IgG or IgM) to a microbial antigen, or binding of pentraxins like C-reactive protein (CRP) and pentraxin III to specific target molecules. By the mechanism of antibody-antigen mediated classical pathway activation, the complement system is linked to the adaptive immune system. The lectin pathway is activated after recognition of carbohydrates on the microbial surface by mannose binding lectin, and also L-ficolin (or ficolin 2) and H-ficolin (or ficolin 3) [1]. The alternative pathway has no specific molecule that aids in activation of this route. Instead it is started up by spontaneous binding of C3 to foreign surfaces in the absence of membrane-bound complement inhibitors. All human cells possess these inhibitors while the surface of most pathogens lacks these proteins. In this way, it is possible for the alternative pathway to discriminate between self and non-self. Of note, the alternative pathway is also particularly important for amplification of complement activation after deposition of C3 on a pathogen by any of the other two initial pathways of complement activation [2].

Elimination of pathogens occurs after cleavage and activation of C3 to C3b and C5 to C5b, key molecules of the complement system. Most pathogens are eliminated by opsonisation with C3b that promotes phagocytosis. C3b bound to a pathogen can be recognised by complement receptors on phagocytic cells in the blood, liver and spleen, and after recognition, phagocytosis can occur. This mechanism is especially important for Gram-positive and encapsulated bacteria which have a thick cell wall. Thus, deficiency of C3 is associated with serious bacterial infections. Gram-negative bacteria, which have an outer membrane and a thin cell wall, can be killed directly by the lytic effect of the membrane attack complex (MAC). The MAC is a macromolecule composed of C5b, C6, C7, C8 and multiple C9 molecules. The MAC penetrates the bacterial cell membrane, punches a hole, and after this lysis of the bacterium will occur. This mechanism of bacterial complement lysis is principally important for killing of Neisseria species.

Cleavage and activation of C3 and C5 occurs by so-called convertases. The classical pathway C3 convertase - C4b2a - is generated when C1q is bound by antibody-antigen complexes and activating C1r/C1s. Subsequently, C4 and C2 can activate and cleave both C4 and C2. The lectin pathway forms the same C3 convertase, and here C4 and C2 are activated when binding of MBL to pathogens triggers the MBL-associated serine proteases (MASPs). The alternative pathway has another C3 convertase, called C3bBbP. This enzyme is formed after binding of factor B to C3b, cleavage of factor B by factor D and stabilization of this complex by factor P. The C5 convertases (C4b2a3b for classical and lectin pathway and C3bBbBbP for the alternative pathway) are formed when a second C3b-molecule binds covalently to the C3 convertases.

The complement system signals danger by the actions of the anaphylatoxins: C4a, C3a and C5a. These are small peptide rem-
nants that arise after cleavage of C4, C3 and C5. In particular C5a has been shown to be a potent pro-inflammatory mediator, whereas C3a has diverse effects also acting anti-inflammatory and possibly bactericidal [3, 4]. Various immune competent cells like granulocytes and mast cells, possess receptors for these anaphylatoxins. The anaphylatoxins exert their actions by ligation of these receptors. In this way, the complement system is also linked to the cellular part of the innate immune system. The specific actions of C5a are discussed below.

Unwanted or excessive activation of the complement system may cause damage to the host. Therefore, the body is equipped with complement-regulatory proteins. The most important are C1 inhibitor that prevents excessive activation of the classical and lectin pathways (5), C4-binding protein that prevents formation of C4b2a, and factor H and I that prevent alternative pathway activation. In addition, host cell-membranes are protected by membrane-bound regulators: Complement Receptor 1 (CR1), membrane cofactor protein (MCP) and decay accelerating factor (DAF). These membrane proteins prevent blood and endothelial cells against C3b formation. In addition, CD59 is present on most cells to protect against insertion of the lytic C5b-9 (MAC) complex. The anaphylatoxins are rapidly inactivated by the carboxypeptidases when they are released in the systemic circulation. Figure 1 gives a simplified overview of the complement system.

**Defence**

Because the complement system is crucially important for the elimination of certain pathogens, inherited or acquired deficiency for a complement protein will predispose to bacterial infection or sepsis.

In animal studies, it has been shown for nearly all complement components - i.e. C1, C2, C3, C4, C5, factor B and factor D - that deficiency leads to either increased susceptibility to infection and/or increased virulence of bacteria [6-9].

In humans, deficiency for C1q, C4 and C2, the proximal components of the classical and lectin pathways, is associated with infections by encapsulated bacteria such as *S. pneumoniae* and *N. meningitidis*. In addition, patients with classical pathway deficiencies are especially prone to developing auto-immune diseases such as systemic lupus erythematosus (SLE). Common pathway deficiency (C3) leads to a severe immune deficiency and predisposes to infections with a wide range of Gram-positive, Gram-negative and other infections. Patients with deficiencies for the alternative pathway and the terminal complement components C6-C9 are at risk particularly for infections with *Neisseria meningitidis*. Because complement deficiencies are rare, with prevalences reported to be < 0.1% [10], they may be of minor importance in daily clinical practice. Yet, they underscore the importance of complement in defence against infections.

Deficiency for MBL, the initiating molecule of the lectin pathway of complement is much more common, with approximately 5-10% of the population being completely deficient for this protein and over 35% partially deficient [11]. MBL deficiency is reported to be associated with pneumococcal and meningococcal infection [12, 13] but overall, only a relatively small number of these infections can be explained by MBL-deficiency. Because of the redundancy of the immune system, MBL-deficiency is probably more important in situations where the immune response is compromised by other mechanisms such as in young children [14], chemotherapy induced neutropenia [15], other immune deficiencies [16], or in critically ill patients (see below).

A number of observations have highlighted the consequences of MBL-deficiency in patients with sepsis admitted to the ICU. First, it appears that patients with sepsis admitted to the ICU have a higher incidence of MBL-deficiency than normal controls or patients with the systemic inflammatory response syndrome (SIRS) in the absence of infection [17-19]. This indicates that MBL-deficiency predisposes to serious infections needing intensive care treatment. Likewise, MBL-deficiency is also linked to a higher number of infectious complications in patients with cancer following major surgery [20, 21].

Second, recently it was found that low MBL-plasma concentrations predispose to poor outcome in sepsis and septic shock, although this effect was only evident in patients with a normal MBL-genotype [22]. In addition, in paediatric patients with sepsis admitted to the ICU, low MBL plasma levels were associated with more severe disease in patients with sepsis [19]. Thus, MBL-deficiency may be associated with a poor outcome in patients with sepsis admitted to the ICU. Interestingly, this may also be the case for patients without infection admitted to the ICU; as it was recently found in a trial assessing the effect of intensive insulin treatment on a surgical ICU unit, that low MBL plasma concentration in the control group, but not in the patients with intensive insulin treatment was linked to mortality [23]. Possibly this is related to the development of more infectious complications in the patients with low MBL plasma concentrations. In sum, in critically ill patients, there is mounting evidence that MBL-deficiency predisposes to infectious complications and is associated with an increased risk for fatal outcome [24].

The role of complement in the defence against infections is indisputable; without it we would succumb to infectious diseases. The complement system is designed to function locally - on the pathogen at the site of infection - in a highly controlled way.

In conditions when the control fails, systemic and insufficiently controlled activation of the complement system can be harmful and disrupt homeostasis; this is the case in sepsis and septic shock. Therefore, the complement system has been termed a ‘double-edged sword’ in the pathogenesis of sepsis.

**Complement System**

![Diagram of the complement system](image-url)

Figure 1. A simplified overview of the complement system.
Figure 2 Actions of C5a of importance during the development of sepsis and septic shock

Damage
The first study to document increased complement activation in sepsis in humans was performed over 30 years ago [25]. Two years later, the same group showed that the alternative pathway was important for this activation [26]. In these studies, complement activation was seen in patients with septic shock, but not in patients with uncomplicated bacteraemia. It was concluded that complement activation in concert with activation of other mediators could play a critical role in the pathogenesis of shock. Subsequent studies showed that increased activation of complement was associated with a poor outcome [27-29], development of shock [30] and the adult respiratory distress syndrome [31].

In the above studies evidence was found for classical and alternative pathway-dependent mechanisms of complement activation during sepsis. It should be noted, however, that in these studies activation of the lectin pathway (only discovered in the 1990s) was indistinguishable from classical pathway activation. Recent evidence from in-vitro and animal models also points to lectin-pathway dependent complement activation during sepsis [32, 33].

It is likely that the pathogen itself is responsible for the systemic activation seen during septic shock; it has been shown that in-vitro, whole blood substantial complement activation is seen after stimulation with different bacteria [32, 34], at concentrations relevant to the situation during septic shock. However, several other mechanisms can also contribute to activation of complement. First, thrombin and fibrin can also activate complement [35] and both proteins are formed in abundance during disseminated intravascular coagulation (DIC). Second, during septic shock, ischaemia-reperfusion (I-R) injury occurs, and activation of complement is an important pathogenic event in I-R [36].

Excessive activation of the complement system with formation of the anaphylatoxins is deleterious to the host. C5a is the best studied anaphylatoxin in sepsis, and a large body of data from animal models indicates that uncontrolled, systemic activation of C5a during sepsis is harmful. Blockade of C5a activity with monoclonal inhibitory antibodies against C5a or C5 in caecal ligation and puncture (CLP)-induced shock in rats has a protective effect [37, 38], and attenuates the development of multi-organ failure [39]. In addition, lung injury in mice induced by administration of cobra venom factor, an activator of complement, is mediated by C5a [40]. In primates receiving a lethal dose of E. coli, anti-C5a protects against early mortality, and inhibits the development of the adult respiratory distress syndrome and shock [41]. Thus, C5a plays a critical role in the development of organ failure during sepsis.

In the human sepsis and septic shock, C5a has been studied and relates to disease severity. C5a is high in sepsis and septic shock [42, 43], normalizes when the patient recovers, and remains high if patients fail to respond to treatment [43, 44]. In addition, soluble terminal complement complexes (sTCC or sC5b-9), that indicate C5a generation, are also increased in patients with septic shock, and correlate with mortality [28, 44].

Because of the important role of C5a in the pathophysiology of sepsis, numerous studies have addressed the mechanism by which C5a can induce damage. It is now evident that during sepsis, C5a exerts various separate but intertwined activities. First, C5a induces the synthesis of other mediators such as the cytokines and activates the coagulation cascade, which are both crucial factors in the development of organ damage during sepsis [45, 46]. Second, granulocytes and endothelial cells are excessively activated, leading to upregulation of pro-inflammatory molecules, adhesion of granulocytes to endothelium, release of oxygen radicals and activation of coagulation [32, 34, 47, 48]. Third, C5a has a direct effect on vasopermeability and vasodilatation [49] and can induce cardiac dysfunction [50], events that are thought to be highly important in the development of shock. Finally, C5a impairs the bactericidal activity of neutrophils [37, 51] which may counteract the host defence against infections. It is likely that these activities in concert are responsible for the harmful effect of C5a activation during sepsis (Figure 2).

The role of the other anaphylatoxins and the MAC in human sepsis is less clear. C3a has bactericidal activities [4], but in contrast to C5a it is thought to have an anti-inflammatory role during sepsis [5]. The MAC is possibly related to lytic damage during sepsis [52]. In addition, the MAC and C3b aid in the induction of apoptosis and the removal of apoptotic cells during inflammatory reactions [53, 54].

Thus, uncontrolled systemic activation of the complement system plays an important role in the development of damage during sepsis, and it is likely that most of these effects are mediated by the anaphylatoxin C5a.
Therapy
Because the evidence from animal models that inhibition of complement activation might be helpful, and the observations in humans that excessive complement activation during sepsis is associated with severe disease and fatal outcome, modulation of complement activation during sepsis has been attempted in humans.

C1 inhibitor that inhibits complement activation by classical and lectin pathway and additionally impedes activation of the contact and kinin systems [5, 55] has been evaluated in a small randomized controlled trial. In this trial involving 40 patients with sepsis, treatment with C1 inhibitor had a beneficial effect on renal function and effectively inhibited activation of classical pathway, the contact system and granulocytes [56, 57]. However, no effect on outcome was observed. In addition, two case reports showed a possible beneficial effect of C1 inhibitor in patients with sepsis and streptococcal toxic shock syndrome [58, 59]. It must be noted however that the classical and lectin pathways are not the only pathways activated during sepsis, and that C1 inhibitor administration in primates suffering from lethal E. coli sepsis had only a limited effect on terminal pathway activation [60].

One important problem of complement-inhibitory therapies aimed at inhibiting initial pathway (i.e. classical, lectin or alternative) activation is that they also have a disadvantageous effect on the host defence. This might be of less importance in the early phase of the disease, when patients are treated with antibiotics and they have an excessive inflammatory response, but can be expected to be harmful later on in the course of their intensive care treatment when multiple organ failure may develop and infectious complications lie in ambush. Therefore, it would be more attractive to inhibit the effects of excessive complement activation without impairing elimination of bacteria, i.e. leaving intact the generation of C3b and the C5b-9 complex. In the light of the crucial role C5a plays in the pathogenesis of sepsis and septic shock, selective inhibition of C5a or the C5a receptor is a promising option. By selective inhibition of C5a, most of the damaging effects of complement activation can be prevented without significantly impairing the host defence systems [32, 61]. Interestingly, in a large randomized placebo controlled trial studying the effect of the C5-inhibitor pexelizumab on mortality and myocardial infarction after CABG, the incidence of septicaemia was significantly reduced, although there were more cases of pneumonia [62].

Although inhibition of C5a may be an attractive approach to diminish the devastating effects of septic shock, it should be noted that the pathogenesis of sepsis and septic shock is complex and involves multiple mediators at multiple time points. Thus, therapies aimed at modulating the course of sepsis would most likely require a multi-target approach and should attack mediators upstream in the inflammatory reaction, of which C5a indeed is one of the candidates.

Summary and conclusions
The complement system is aimed at recognizing pathogens and eliminating them. In addition, it provides danger signals. Complement is essential in the defence against certain infections; complement deficiency, MBL being the most frequently occurring, predisposes to serious infections. On the other hand, during sepsis and septic shock, excessive systemic activation of complement contributes to the development of shock and organ failure. The anaphylotoxin C5a plays a key role in the pathogenesis of sepsis. Future therapies aimed at modulating the complement system should target this molecule or its receptor.

References


