# Complement in sepsis—when science meets clinics

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Sepsis as life-threatening organ dysfunction caused by microorganisms represents a dreadful challenge for the immune system. The role of the complement system as major column of innate immunity has been extensively studied in various sepsis models, but its translational value remains in the dark. Complement activation products, such as C3a and C5a, and their corresponding receptors provide useful diagnostic tools and promising targets to improve organ function and outcome. However, a monotherapeutic complement intervention irrespective of the current immune function seems insufficient to reverse the complex sepsis mechanisms. Indeed, sepsis-induced disturbances of cross talking complement, coagulation, and fibrinolytic cascades lead to systemic 'thromboinflammation', ultimately followed by multiple-organ failure. We propose to reliably monitor the complement function in the patient and to re-establish the immune balance by patient-tailored combined therapies, such as complement and Toll-like receptor inhibition. Our working hypothesis aims at blocking the 'explosive' innate immune recognition systems early on before downstream mediators are released and the inflammatory response becomes irreversible, a strategy that we name 'upstream approach'.

**Keywords:** C3a; C5a; complement activation; sepsis; therapy; Toll-like receptors

Sepsis comes from the Greek word 'sepein' meaning 'to rot' reflecting an extreme process of irreversible tissue damage. Others defined sepsis as intoxication of the blood, emphasizing the dynamic process of sepsis with exposure to pathogen-associated molecular patterns (PAMPs) generated and released by microorganisms including bacteria, worms, parasites, fungi, and viruses. In the last three decades, sepsis was defined as a rather general immune pathophysiologic reaction with focus on the hemodynamic and pulmonary response, as well as on temperature and leukocyte count, clinically known as systemic inflammatory response syndrome (SIRS) to verifiable bacteria [1]. Only in the last decade, sepsis has been proposed as life-threatening organ dysfunction caused by a dysregulated host response to infection [2]. Irrespective of the definition and modern diagnostic algorithm and intensive care treatment, sepsis remains associated with a high morbidity and hospital mortality rate reaching more than 25% in cases of severe sepsis [3]. Thus, sepsis represents a major challenge for both clinicians and scientists.

As for the underlying mechanisms, classical PAMPs originated from Gram-negative or Gram-positive

**Abbreviations**
CLP, cecal ligation and puncture; LPS, lipopolysaccharides; MODS, multiple-organ dysfunction syndrome; PAMP, pathogen-associated molecular pattern; SIRS, systemic inflammatory response syndrome; TLR, Toll-like receptor.

bacteria, such as lipopolysaccharides (LPS) or lipoproteins, respectively, were previously in the focus as triggers for sepsis. However, PAMPs from worms, parasites, fungi, and viruses can also induce sepsis. In all cases, innate immunity comprising cellular and fluid-phase defense systems is challenged by microorganisms invading from external (e.g., skin) or internal (e.g., gut) surfaces via dysfunctional barriers to otherwise sterile areas of the body [4]. The innate immune system orchestrates a highly interactive response against microbes with multiple instruments such as neutrophils, monocytes and macrophages, dendritic cells, innate lymphoid cells, and the complement system as main players for an effective defense [5]. However, although designed as protective systems, the response may become inappropriate and excessively activated, leading to an unbalanced response that puts the patient at risk of an overwhelming inflammation incompatible with life.

## The complement system

Complement is part of the host innate defense with a number of biological effects, many of which contribute to the inflammatory reaction mainly by activation of cells like leukocytes and endothelial cells. Previously, and still correct, complement is regarded as a protection system against infection, but the recent decades have shown that complement contributes a number of other host functions in maintaining the internal tissue homeostasis [6], including tissue regeneration and renovation, embryogenesis, neuronal junction regulation in addition to cross talk with a number of other biological systems, including the TLRs [7]. However, the system is a double-edged sword since improper, enhanced, or uncontrolled activation is disadvantageous and potentially harmful and may be even lethal for the host in case of septic shock [4].

Complement comprises approximately 50 soluble and surface bound proteins, acting together in a highly specific manner and is kept under strict control by regulatory proteins (Fig. 1). The complement system can be activated through three pathways (upper part of Fig. 1), all converging to the cleavage of C3 to generate C3a and C3b (middle part of Fig. 1). The classical pathway (CP) is typically activated by antibodies, but also pentraxins including C-reactive protein (CRP), serum amyloid P component (SAP), and pentraxin 3 (PTX3) can activate C1. The lectin pathway (LP) is activated through recognition of carbohydrates by mannose-binding lectin (MBL), and other collectins as well as ficolins. Furthermore, LP activation may be mediated through IgM antibodies, for example, by

those directed against damaged self-antigens. The alternative pathway (AP) is activated by foreign or damaged own cells, facilitated by the continuous spontaneous hydrolysis of C3. AP also has an important function in the complement system providing an amplification loop enhancing C3 activation independent of which pathway has been initially activated. This effect is mainly due to properdin (FP), the only positive regulator in the complement system, which stabilizes the C3 convertase. Activation of C3 leads to formation of a C5 convertase, cleaving C5 into C5a and C5b.

The anaphylatoxins C3a and C5a bind to the receptors C3aR, C5aR1, and C5aR2, leading to downstream production of inflammatory mediators (lower left part of Fig. 1). C5b initiates the formation of the terminal C5b-9 complement complex (TCC), which forms the membrane attack complex if inserted into a membrane (bottom part of Fig. 1). This may lead to lysis of bacteria and cells or in sublytic doses to activation of cells. The cleavage and inactivation of C3b generate iC3b, which binds to complement receptors CR3 (CD11b/CD18) and CR4 (CD11c/CD18), facilitating phagocytosis, oxidative burst, and downstream inflammation (right part of Fig. 1). The complement system is tightly regulated by soluble inhibitors (marked in yellow in Fig. 1), including C1-inhibitor (C1-INH), factor H (FH), factor I (FI), C4b-binding protein (C4BP), anaphylatoxin inhibitor (AI), vitronectin (Vn), and clusterin (Cl), keeping the continuous low-grade activation in the fluid phase in check. Host cell membranes are equipped with a number of inhibitors to protect them against attack by complement (right part of Fig. 1), including membrane cofactor protein (MCP;CD46), complement receptor 1 (CR1;CD35), decay accelerating factor (DAF;CD55), controlling C4 and C3 activation, and CD59 protecting against final assembly of the C5b-9 complex.

## Investigation of complement activation mechanisms in *ex vivo* sepsis 'surrogate' models

Sepsis is a clinical condition, requiring the whole body of the host to interact with the microbes, which are growing in the circulation and infiltrating organs through a disturbed vascular endothelium. Since *ex vivo* models do not fulfill the criteria for the term sepsis as a multi-organ response, they are not termed sepsis models, but rather models for bacteria-induced inflammation. These can be highly reductionistic, studying the interaction of the microbe with specific cell population in purified systems. Such models are
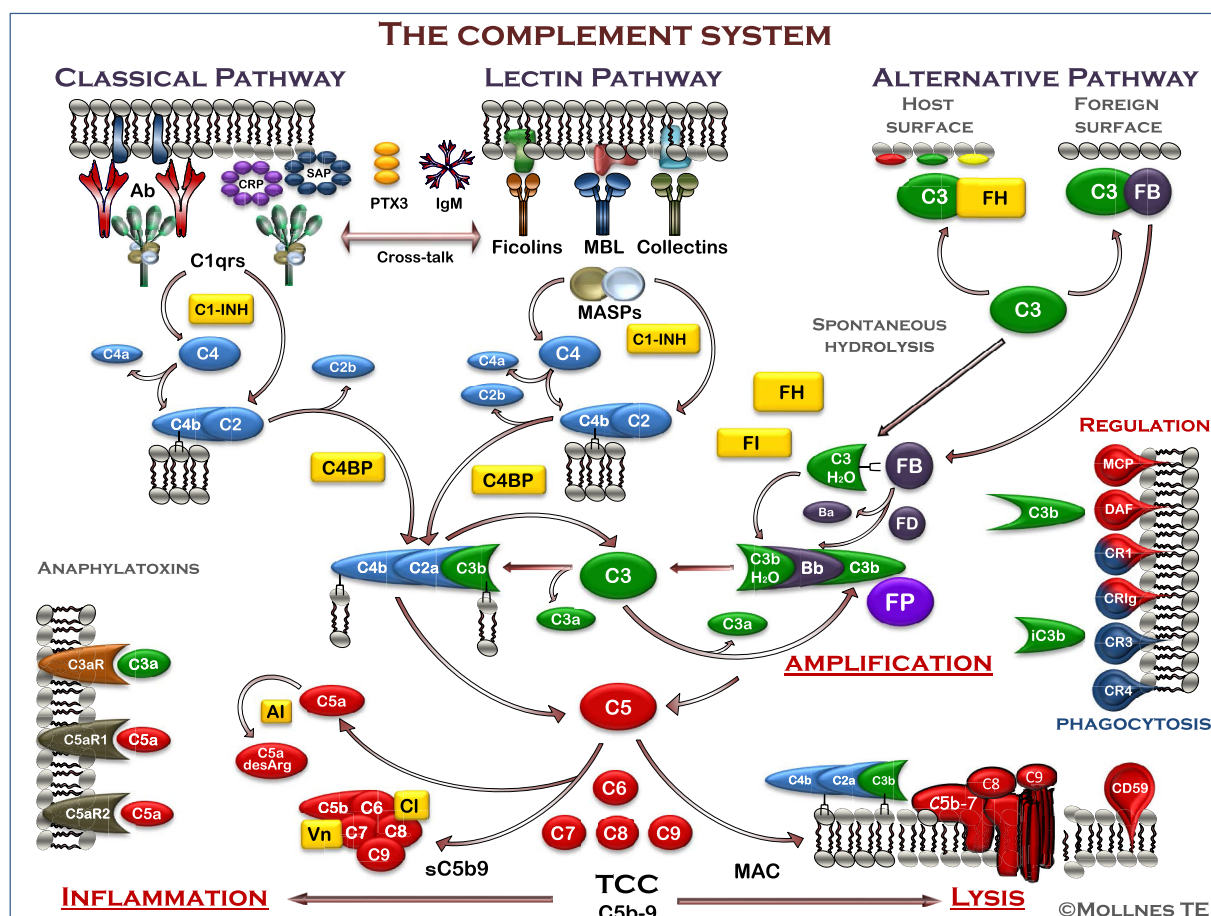
**Fig. 1.** Overview over the complement system. The content of the figure is described in detail in the section entitled 'The complement system'.

valuable to study ligand–receptor interactions and the subsequent intracellular signaling pathways. Targeting specific molecules in these signal pathways are useful to test potential therapeutic approaches both by blocking (antagonists) and by enhancing (agonists) the actual signaling pathway. Various microbe–cell interaction models both with single cells and more complex cell cultures have been reviewed [8].

We have developed a more holistic *ex vivo* model based on freshly drawn human whole blood, which is anticoagulated with lepirudin, which is a highly specific thrombin inhibitor [9]. Traditional anticoagulants like EDTA and citrate bind calcium, thus precluding them for being used when that aim is to explore the interaction between the various biological systems of which most are dependent on free calcium. Heparin interferes not only with many steps of the coagulation cascade, but also with the complement system and should be used with great caution. Our lepirudin-based

model inhibits only thrombin, the second last step of coagulation. Thus, despite the limitation of the model that the role of thrombin cannot be evaluated, it is as close to a physiological system as is possible to come using whole blood.

The role of complement in the inflammatory reaction in human whole blood has been extensively studied in this model using both Gram-negative and Gram-positive bacteria. Various complement inhibitors as well as whole blood form otherwise healthy C5-deficient individuals were used to study the effect of complement activation on the inflammatory reaction including leukocyte and platelet activation, cytokine release, oxidative burst, receptor expression, and arachidonic acid production [10]. Complement activation, in particular of C5 with release of C5a and subsequent binding to its receptor C5aR1, was responsible for a number of the secondary inflammatory mediators, in particular those related to neutrophil activation like

oxidative bust and granula release, receptor expression, and IL-8 release. The effect of the rest of the cytokine panel was less complement driven. This led us to search for the role of another important upstream recognition system of innate immunity, the Toll-like receptor (TLR) system. CD14 is an important coreceptor for several TLRs, including TLR4 and TLR2, both of which are important sensors of PAMPs [11]. Thus, we used the same whole blood model and inhibited the same panel of inflammatory markers induced by the same bacteria using an anti-CD14 antibody [10]. We then found a differential pattern with the most extensive effects on the typical pro-inflammatory cytokines including IL-6, TNF, and IL-1β. Most importantly, we found that a combined inhibition of C5 and CD14 virtually abolished the whole inflammatory reaction.

Based on these data, we developed the hypothesis that dual blockade of both complement (e.g., C3, C5, or C5aR1) and the TLR molecule CD14, binding LPS captured by LPS-binding protein (LBP) in plasma bringing it to TLR4, was a potent way of attenuation the overwhelming inflammatory response induced by Gram-positive as well as Gram-negative bacteria [12]. In a number of studies, we showed that this combined inhibition was a promising strategy for future therapy, reviewed in [13,14], paving the way for animal experiments described below. In order to investigate how potent this combined regimen was, we performed a microarray study of human whole blood exposed to *Escherichia coli* (*E. coli*) in the absence or presence of the combined treatment approach [15]. In this model, 2335 genes significantly responded to *E. coli.* by on average 80%. Combined inhibition significantly reversed 70% of these transcriptional changes by an average of 80%, underscoring an extensive and broad-acting effect of this inhibition in a bacterial model.

## Determination of the effects of complement modulation during sepsis in animal models

### Rodents

Multiple models exist for the scientist to simulate systemic inflammation following an infectious insult. However, in real world, the clinician usually faces a more complex immune pathophysiology induced by other situations than just an artificial LPS intoxication or inoculation of a single specific bacterium. To simulate sepsis in a clinically more relevant manner, cecal ligation and puncture (CLP) in rodents [16] or cecum incision in pigs [17] (see below) seems rather reliable and considered currently as a golden standard for

translational meaningful polymicrobial sepsis research [18]. In rodent CLP sepsis, acute-phase protein, transferrin, and C3 production by the liver was increased as early as 16 h after CLP induction as determined in ex vivo hepatic perfusion analyses [19] and remained more than twofold even 96 h after induction of sepsis [20]. Systemic complement activation with generation of the complement activation products C3a and C5a was shown in many studies in rodent sepsis [21]. However, blockade of C3 or C4 by genetic deficiency resulted in impaired endotoxin clearance and worsened the septic course and outcome in an LPS intoxication model [22]. Furthermore, C3 knockout mice were also very susceptible to CLP-induced sepsis [23]. In contrast, C5 inhibition by antibodies and C6 deficiency in rats significantly improved animal survival [24]. Another study reported some protection in C3-deficient mice undergoing CLP sepsis but no survival advantage of C5-deficient mice [25]. In line, a recent CLP sepsis study revealed that upregulation of microRNA-574 which gen-targets and downregulates C3, attenuated septic lung injury and reduced sepsis-induced stress of the endoplasmic reticulum [26].

There are several studies indicating improved cellular function, organ performance, and survival of CLP sepsis mice by several inhibition strategies of the interaction between C5a and the corresponding receptor C5aR1 or C5aR2 [27–30]. Of note, in severe sepsis, synchronic inhibition of both, C5aR1 and C5aR2, revealed a survival benefit over single blockade strategy [28].

For blockade of the C3aR in CLP sepsis, little is known. One study showed in murine endotoxin shock an enhanced mortality rate when C3aR was absent [31]. Another study in LPS-induced shock with systemic inflammation demonstrated an early enhanced lethality rate of C3aR-deficient mice, but after a 72-h observation period, no changes could be detected between the wild-type and C3aR-deficient littermates [32]. In experimental meningococcal sepsis, a differential role for the C3aR and the C5a receptors has recently been reported: whereas C5aR1 and C5aR2 aggravated the disease immune pathology, C3aR was rather protective [33]. Thus, C3a seems to reveal some protective effects, possibly by activating the hypothalamus–pituitary–adrenal axis and by inhibition of neuronal cell death [32].

In striking contrast, C5a seems to induce all classical signs of inflammation and has been proposed to be harmful when excessively produced [34]. Mechanistically, C5a generation during sepsis as a most potent chemotactic factor for neutrophils can switch the endothelium from antiadhesive to pro-adhesive for inflammatory cells by upregulation of various selectins

[35]. C5a can also result in the disruption of the endothelial barrier by neutrophil protease-mediated degradation of VE cadherin [36]. Furthermore, C5a can transform leukocyte surfaces to a pro-coagulatory platform [37,38]. Upon recruitment, C5a can activate leukocytes to mount an oxidative burst, which is not only microbicidal but also host-attacking. However, during advanced sepsis and upon exposure to excessive C5a, neutrophils become 'paralysed' and may not response to chemotactic signals. Their NADPH oxidase activity will be lost by translocation defects of key activation enzymes (e.g., p47phox) and lead to an impaired phagocytotic activity and bacterial clearing [27,39]. Therefore, bacteria can further expand, drive sepsis progression, and worsen clinical outcome [27].

Sepsis in the clinical setting is currently defined as (multiple) organ dysfunction [2]. In this context, C5a has detrimental effects to several organs as previously shown [40]. During rodent CLP sepsis, pulmonary gas exchange was compromised, liver enzyme levels elevated, urine output and glomerular filtration rate reduced with ultrastructural changes of the glomerular filter, and lactic acidosis present. All these pathological changes could be prevented by blockade of systemic C5a [17]. Furthermore, sepsis-induced coagulopathy could be alleviated by anti-C5a therapeutic strategies [41]. In addition, applying a C5a-neutralizing mirror image (L-) aptamer (C5a-Spiegelmer; an RNA fragment binding and blocking C5), mice with CLP-induced sepsis exhibited less signs of gut barrier dysfunction and multiple-organ failure even when

applied 6 h after the infectious insult [29]. With the same C5a-neutralizing strategy, pneumococcal pulmonary sepsis in mice could also be improved [42]. Using anti-C5a antibodies, the delayed application of the immune modulation could last up to 12 h after CLP and still resulted in an improved outcome [43]. Remarkably, C5a seems also to be involved in development of septic cardiomyopathy with dysfunction of cardiomyocytes, which could be almost normalized by neutralizing C5a [44].

Taken together, C5a inhibitory strategies in small animals with sepsis simulation seem promising on a cellular, immune, and multiple-organ levels and, therefore, have been translated to higher species so as to assess its potential for clinical application (Fig. 2).

## Large animals

In contrast to rodents, very few studies have been performed using complement inhibitors in large animal sepsis studies. Some reports on C1-inhibition have been published, but since the C1-inhibitor is not a specific complement inhibitor, but inhibits all plasma cascade systems, we have not included these here. It is well known that the pathophysiology caused by C1-inhibitor deficiency is due to bradykinin formation from the contact activation system [45].

We established a pig model of intravenous *E. coli* sepsis with the aim of investigating a broad panel of physiological, inflammatory, and hemostatic parameters [46]. At that time, we already had established the
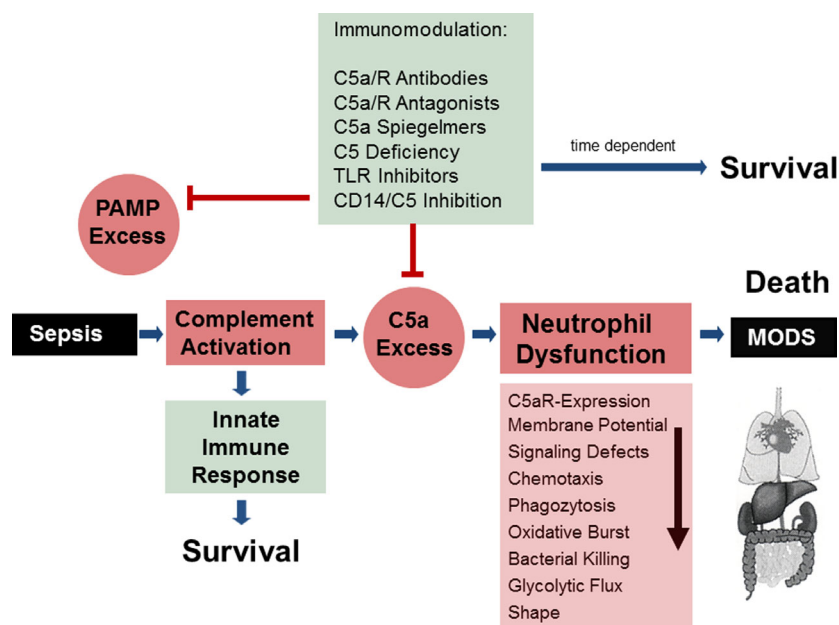


**Fig. 2.** Possible complement modulatory strategies for excessive complement activation and the subsequent signs of cellular and organ dysfunction during sepsis. Blue arrows: activation/stimulation; red arrows: inhibition.

complement and CD14 dual blockade. Thus, we investigated the efficacy of blocking C5 alone or in combination with an anti-CD14 antibody. To block C5, we used Ornithodoros moubata complement inhibitor (OmCI), also known as coversin. Anti-CD14 (mAb MIL-2) was modified by making it a chimeric IgG2/4, which did not show diverse effects due to Fc-mediated effector functions, but completely neutralized CD14 [47]. Of interest, C5 blockade not only completely inhibited complement terminal activation, but also abolished leukotriene B4 formation, and significantly reduced granulocyte tissue factor expression. Formation of thrombin–antithrombin complexes and formation of TNF and IL-6 were efficiently inhibited by OmCI alone, supporting a cross talk with coagulation, that is, 'thromboinflammation'. When anti-CD14 was added together with OmCI, these mediators were virtually abolished, indicating the potency of the dual inhibition. Additionally, the combined therapy attenuated the formation of plasminogen activator inhibitor-1 ($P < 0.05$), IL-1β, and IL-8, increased the formation of IL-10, and abolished the expression of wCD11R3 (CD11b) and the fall in the neutrophil cell count. Finally, the combined treatment also improved the cardiopulmonary functions by delaying the increases in the heart rate and mean pulmonary artery pressure. We then investigated the effects of the combined inhibition on the local inflammatory response in liver, kidney, lung, and spleen from these animals [48]. All cytokines were reduced in all organs and the therapy attenuated upregulation of C5aR in the heart and lungs, which could explain the beneficial effects on the cardiopulmonary functions.

Similar to the model described above, we tested the dual inhibition in Neisseria meningitis sepsis by intravenous infusion of escalating doses of bacteria [49]. The dual inhibition markedly attenuated the cytokine storm, which developed rapidly in the untreated animals, similar to the human meningococcal sepsis, by significantly inhibiting interferon-gamma, TNF, IL-8, IL-10, IL-12p40, and granulocyte CD11b (CR3) expression. Thus, inhibition of C5 and CD14 may be beneficial in treatment of Gram-negative sepsis in general.

In clinical medicine, polymicrobial sepsis is common, frequently presented with several Gram-negative and Gram-positive bacteria, typically seen in sepsis originated from peritonitis. As described above, we have tested mice with the CLP model of sepsis and found that dual inhibition both increased survival and decreased inflammation as compared to single inhibition. Though, this mouse model is regarded as a highly relevant model for sepsis, 'mice are not man'

[50]. Thus, in order to move closer to the clinic, we developed a peritonitis model in pigs by making a 2 cm cut in cecum [17]. Peritonitis developed rapidly and led to polymicrobial severe sepsis, septic shock, and many deaths throughout the 8-h observation period. Treatment was performed with the same inhibitors of C5 and CD14 as described for the Gram-negative bacteria studies. Dual inhibition improved cardiopulmonary function, reduced the cytokine storm, and, most importantly, significantly improved survival. Of the 12 animals in each group, nine survived in the treatment group and four in the nontreated group.

Even closer to humans are nonhuman primates. In a primate study of E. coli sepsis, an anti-C5a antibody was shown to attenuate the septic shock and reduced leukocyte-mediated lung edema [51]. In a well-established baboon model of intravenously induced E. coli sepsis, inhibition of C3 by the small molecule compstatin, the authors investigated the effect of complement inhibition on hemostasis [52]. C3 inhibition reduced leukopenia and thrombocytopenia and lowered the accumulation of macrophages and platelets in organs. It decreased the coagulopathic response by downregulating tissue factor and PAI-1, diminished global blood coagulation markers like fibrinogen, fibrin degradation products, and APTT, and preserved the endothelial anticoagulant properties. Furthermore, compstatin effectively reduced tissue damage as evaluated by histopathology.

We followed this track by investigating the effect of C5 inhibition in the same baboon E. coli sepsis model [53]. C5 cleavage was blocked by RA101295, a 2-kDa macrocyclic peptide. RA101295 reduced the E. coli-induced 'oxidative burst', as well as leukocyte activation, without affecting phagocytosis. RA101295 treatment reduced the LPS content in plasma, implying reduced complement-mediated bacteriolysis, whereas treated animals showed slightly improved bacterial clearance during the bacteremic stage compared with controls. Treatment also improved consumptive coagulopathy, preserved endothelial anticoagulant and vascular barrier functions, and reduced cytokines. Overall, RA101295 treatment was associated with significant cardiovascular improvement and organ protection and, most importantly, markedly reduced mortality as four of five animals survived in this 100% lethal model. We therefore conclude that inhibition of C5 cleavage during the bacteremic stage of sepsis could be an important therapeutic approach to prevent sepsis-induced inflammation, consumptive coagulopathy, and subsequent organ failure and death.

# Translational approaches of complement monitoring and modulation in clinical sepsis

Various clinical studies have demonstrated in patients with severe sepsis a robust complement activation with enhanced concentrations of complement factor B [54] and generation of C3a, C4a, and C5a. The anaphylatoxin production in particular seems to be associated with a bad outcome [55–62]. In patients suffering from necrotizing soft tissue infections with or without septic shock, complement activation was evident and high levels of the C4c/C4 ratio, C3bc, and the C3bc/C3 ratio were associated with an enhanced long-term mortality [63]. Patients with severe tissue trauma also exhibited enhanced C3a and C5a plasma levels especially when they developed septic complications [64,65]. The systemic concentrations of C3 and C5 dropped whereas C3a and C5a significantly increased early during sepsis and normalized when successfully treated [66].

C3a was the best variable to differentiate between sterile SIRS and sepsis in humans with a maximal sensitivity of 86% and a specificity of 80% [67]. For C5a, the discriminatory power to reliably diagnose sepsis versus systemic inflammation or healthy conditions is problematic. Likely, most of the excessively generated C5a is rapidly bound to the almost ubiquitous expressed C5a receptors (C5aR). In contrast, expression of the C5aR on neutrophils seems a rather sensitive tool. Injection of E.coli LPS (2 ng·kg$^{-1}$ body weight) into healthy volunteers resulted among others in a decrease of the C5aR associated with functional cellular impairment [68]. First reports in patients with sepsis indicated that a decreased C5a-C5aR interaction on neutrophils occurred during sepsis development [69]. Meanwhile, determination of C5aR1 or C5aR2 on neutrophils by flow cytometric methods seems a reliable tool for various intensive care patients to early diagnose sepsis with a satisfying sensitivity and specificity [62,70–72]. Human neutrophils, central in the first line of cellular defense, exhibit only minor functional changes when activated by C3a, resulting, for example, in mounting of an oxidative burst [73]. Furthermore, LPS exposure is capable to upregulate C3aR on neutrophils and thereby inducing neutrophil extracellular trap (NET) formation [74].

In striking contrast, presence of C5a results in multiple changes of human neutrophils [75] including depolarization of the membrane potential [76] shape changes [77], phagocytosis, and generation of reactive oxygen radicals (ROS). Furthermore, C5a seems to function as a switch of the pH resulting in intracellular alkalinization followed by enhanced glycolytic flux and production of a lactic acidosis in the extracellular microenvironment of the cells [78]. In accordance, neutrophils isolated from patients with severe sepsis revealed an elevated intracellular pH [78]. Based on all the reported small and large animal studies, where C5a inhibitory strategies revealed some cellular and organ protection and improved outcome it was rational to transfer these results from the scientific platform into the clinics. Thus, an extracorporeal immunoabsorption device to clear LPS, IL-6, and C5a was tested and could successfully reduce systemic C5a levels from ca. 300 to ca. 80 ng·mL$^{-1}$. Moreover, impairment of monocytic function was reversed and some improvement of the organ functions achieved [79]. Furthermore, a sepsis study 'Studying Complement Inhibition in Early, Newly Developing Septic Organ Dysfunction (SCIENS)' has been performed in patients with advanced sepsis using a humanized monoclonal antibody against human C5a (NCT02246595). Although the recruitment phase has been finished, the results are pending.

Taken together, C5aR status on neutrophils, rapidly assessed by flow cytometric methods, may be useful to monitor the risk to develop infectious complications in ICU and sepsis patients [62,70,72]. However, the benefit of C5a inhibitory strategies in human sepsis in clinical reality remains to be proven.

# Future perspectives

## Sepsis-induced multiple-organ dysfunction syndrome

Concerning sepsis-induced multiple-organ dysfunction syndrome (MODS), barrier dysfunction seems a major underlying immune pathophysiological driver. Complement activation products may cause or at least help impair endothelial organ barriers, clinically manifested as capillary leakage syndrome [80,81]. Thus, one important future goal is to modulate the complement in order to treat capillary leakage. Immunoadsorption, hemofiltration, or hemodialysis approaches to remove complement activation products [82–84] may reduce systemic inflammation and thereby result in an improved outcome. Further clinical trials need to address and assess this potential.

Indirect modulation of systemic complement activation may also improve cellular and organ performance and outcome. For example, sIL-1R antagonist attenuated generation of C3a and thrombin–antithrombin complexes, as well as key fibrinolysis markers, during sepsis in humans [85].

Attention should be paid to potential cardiotoxic effects of complement activation products. It has been shown that C3a or C5a generated during sepsis in humans can alter cardiomyocyte function [86,87] and may be directly involved in causing hemodynamic disturbances and a fatal outcome [88]. Here, further research on the proposed cardiocomplement cross talk is required.

Both basic research and clinical research are necessary to better define the role of complement during fungus-induced sepsis. Although considered in general harmful during sepsis [34], C5a may also control early fungal invasion by promoting recognition and clearance of fungi by neutrophils [89]. However, the complement defense is also time-dependent and, in later periods of sepsis, C5a may play a different role [89]. Thus, irrespective of the complement modulatory strategy used, it seems mandatory to monitor actual complement function in accordance to the principle 'measure what your target'. Here, bedside tests are needed to reliably assess actual complement activity or depletion. Rebalancing the activity of complement could be useful as a potential immunomodulatory therapy.

## Summary—specific therapeutic approach in sepsis is to 'look upstream'

Sepsis is a complex spectrum of clinical entities, a big challenge when it comes to clinical trials. Except antibiotics and supportive therapy, there is no specific treatment for sepsis. Due to the diversity of the disease, it is unsurprising that all clinical using specific inhibition of single downstream inflammatory mediators such as TNF, IL-1β, or IL-6 trials has failed [14]. Similarly, also single inhibition of TLR4 (Eritoran) [90] or activated protein C (Xigris) [91] has failed. Stratification of patients is also a big problem: It is vital to determine at which stage of the process leading from infection to septic shock treatment needs to be started. Knowing the cause of sepsis is crucial as well to find an appropriate therapy. Thus, all of these factors should be carefully weighed when designing a treatment regimen for sepsis.

Our hypothesis is to block the complement system in sepsis, but, based on the data presented above, we frankly admit that this is not sufficient. Complement is important, but the other recognition systems are important as well, and a combination of inhibition of other upstream molecules like CD14 in the TLR system seems to be a promising approach [14,92]. During development of sepsis, the innate immune system, including the plasma cascades, gradually reaches a point where it is impossible to save the life of the patient—the so-called 'the point of no return'. These cascades represent undetonated bombs, which may detonate in sepsis. A prerequisite for our hypothesis is that inhibition should be given long before 'the point of no return' is reached. To avoid an explosive sepsis, we need to treat the patients at high risk, ideally before they develop sepsis. High-risk patients include those undergoing abdominal surgery with postoperative peritonitis, or patients with open heart surgery or after major trauma. Treatment should be initiated before development of SIRS and eventually sepsis has occurred, so that the cytokine storm may be prevented.

## Acknowledgements

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