Harmful molecular mechanisms in sepsis

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Abstract | Sepsis and sepsis-associated multi-organ failure are major challenges for scientists and clinicians and are a tremendous burden for health-care systems. Despite extensive basic research and clinical studies, the pathophysiology of sepsis is still poorly understood. We are now beginning to understand that sepsis is a heterogeneous, dynamic syndrome caused by imbalances in the ‘inflammatory network’. In this Review, we highlight recent insights into the molecular interactions that occur during sepsis and attempt to unravel the nature of the dysregulated immune response during sepsis.

The clinical manifestations of sepsis were already known to Hippocrates (460–377 BC), who introduced the term ‘wound putrefaction’. In addition, the Persian ‘father of modern medicine’, Ibn Sina (also known as Avicenna, AD 980–1037), observed that septicemia was usually accompanied by fever. However, it was not until the 18th century that Louis Pasteur linked the decay of organic substances to the presence of bacteria and microorganisms, and Ignaz Semmelweis observed the significant effect of hygienic measures on decreasing the mortality of women during childbirth. In 1914, Hugo Schottmüller laid the foundations for a modern definition of sepsis and was the first to describe that the presence of an infection was a fundamental component of the disease. Decades later, the ideas of Lewis Thomas led to a turnaround in the understanding of sepsis by popularizing the theory that “…it is the [host] response … that makes the disease”.

This theory resulted in a large number of experimental and clinical studies, which eventually shifted the focus of sepsis research from the infectious agent to the host immune response. Finally, the concept entered into daily clinical practice when Roger Bone and colleagues defined sepsis, which involves a complete shut-down of important immune function and metabolic balance and death.

T_{17} cells

A subset of CD4⁺ T helper cells that produce interleukin-17 (IL-17) and that are thought to be important in inflammatory and autoimmune diseases. Their generation involves IL-25 and IL-21, as well as the transcription factors RORγt (retinoic-acid-receptor-related orphan receptor-γt) and STAT3 (signal transducer and activator of transcription 3).

Sepsis

A systemic response to severe infection or tissue damage, leading to a hyperactive and unbalanced network of pro-inflammatory mediators. Vascular permeability, cardiac function and metabolic balance are affected, resulting in tissue necrosis, multi-organ failure and death.

T_{1,2} cells

(T helper 1 cells). A subset of CD4⁺ T helper cells that produce interleukin-2 (IL-2) and IL-15, and that are thought to be important in inflammatory and autoimmune diseases. Their generation involves IL-25 and IL-21, as well as the transcription factors RORγt (retinoic-acid-receptor-related orphan receptor-γt) and STAT3 (signal transducer and activator of transcription 3).

In the past, sepsis was commonly thought to be caused by overactivation of the innate immune system, and the ensuing pro-inflammatory cascade, in response to severe microbial infection or extensive tissue damage (such as caused by burns or multiple injuries). Activation of the complement system and hyperactivation of cellular innate immune responses are associated with an excessive inflammatory response that characterizes sepsis. After being triggered by an overwhelming initial stimulus, neutrophils and macrophages produce and respond to cytokines, chemokines, complement-activation products and other mediators. This pro-inflammatory environment causes the release of powerful secondary mediators (such as lipid factors and reactive oxygen species) that further amplify the inflammatory process. The malfunction of regulatory mechanisms during sepsis can result in a loss of control of inflammation, eventually leading to host damage due to overzealous activation of the inflammatory response.

However, the failure of anti-inflammatory therapies for sepsis in clinical trials raised the question of whether mortality in sepsis actually derives from an uncontrolled pro-inflammatory response. Although some patients die during the initial, hyperinflammatory phase of sepsis, most patients succumb at later time points that are associated with a prolonged immunosuppressive state. Notably, neutrophils can undergo ‘immune paralysis’ during sepsis, which involves a complete shut-down of important intracellular signalling pathways, and dysfunction of the adaptive immune system is also an important contributing factor to the immunosuppression that is observed in the later stages of sepsis. T cells are thought to orchestrate the inflammatory response, particularly CD4⁺ T helper 1 (T_{1,1} cells) and T_{1,2} cells, which have distinct cytokine profiles. During sepsis, the adaptive immune response diverts from an initial T_{1,1}-cell response (characterized by interferon-γ (IFNγ) and interleukin-12 (IL-12) production) to a T_{1,2}-cell response (characterized by IL-4, IL-5, IL-10 and IL-13 production), which can result in profound immunosuppression. The T_{1,1}→T_{1,2} paradigm that describes how T_{1,1} cells interact with other immune cells has recently been expanded with the discovery of T_{1,17} cells, a subset of T_{1,1} cells that produces IL-17 (REF 6). T_{1,17} cells are thought to be important for immunity to microorganisms that are not eliminated by T_{1,1}- or T_{1,2}-mediated immune responses.
Increased levels of apoptosis in lymphocytes and dendritic cells (DCs) further contribute to the suppression of immune responses during sepsis (reviewed in REF 4). In addition to causing a marked decrease in cell numbers, the apoptosis of lymphocytes and DCs contributes to immunoparalysis through the immunosuppressive effects of apoptotic cells. However, different types of immune cell receive different apoptotic signals during sepsis. In contrast to lymphocytes and DCs, the apoptosis of macrophages and neutrophils seems to be unaffected or even increased during sepsis7,8. Whereas the increased apoptosis of lymphocytes and DCs results in severe immunosuppression, which places the patient at risk of nosocomial infections, decreased neutrophil apoptosis increases the bystander damage caused by their pro-inflammatory activity. Recent data indicate that T-cell-mediated suppression of the early innate immune response is required to minimize damage to the host and maximize the host defence response9.

There is now evidence that sepsis is a condition that affects not only the immune system but also other biological systems, such as the coagulation system and the autonomic nervous system (ANS)10-12. In this Review, we describe the interplay between normally host-protective mechanisms that, through amplification or suppression during sepsis, can become instruments of harm. We discuss the mechanisms that initiate dysregulation of the inflammatory response and describe the role of specific inflammatory mediators that act as 'central hubs' to connect the various components of this response. In addition, we describe the pathogenic roles of the plasmatic cascades (the coagulation, fibrinolysis and complement systems) and the recently recognized interactions that occur between them, as well as new insights regarding the influence of the ANS on the inflammatory response. To illustrate the complexity of the inflammatory response in sepsis, we highlight the multidirectional interactions between the various systems that contribute to sepsis pathogenesis in a complex "inflammatory network".

**Initiation of the inflammatory response**

Immune cells express a set of receptors known as pattern-recognition receptors (PRRs) that rapidly initiate host defence responses after detection of tissue damage or microbial infection. The presence of a microbial infection is detected by recognizing conserved pathogen-associated molecular patterns (PAMPs) that are expressed by both invading and innocuous microorganisms. By contrast, immune recognition of damaged tissue is mediated by intracellular proteins or mediators that are released from dying cells. These proteins are known as "alarmins" and, together with PAMPs, are referred to as damage-associated molecular patterns (DAMPs)13. Toll-like receptors (TLRs), which are a subfamily of PRRs, have emerged as crucial receptors for the recognition of DAMPs and initiation of the inflammatory response.

During sepsis, there is a full-blown, systemic activation of immune responses due to the release of very high levels of DAMPs from invading microorganisms and/or damaged host tissue, which leads to the overstimulation of immune cells. As a result, sepsis is accompanied by a markedly imbalanced cytokine response (known as a 'cytokine storm'), which converts responses that are normally beneficial for fighting infections into excessive, damaging inflammation.

**TLR4-mediated recognition of lipopolysaccharide (LPS)**, a well-characterized PAMP that is found in the outer membrane of Gram-negative bacteria, is thought to be an important trigger of the inflammatory response in sepsis14,15. TLR4 forms a receptor complex with CD14 and MD2, the latter of which also has an important role in the recognition of LPS16,17. In addition to LPS, various endogenous ligands for TLR4 have been described, including high-mobility group box 1 protein (HMGB1), which is an important mediator during the late phase of sepsis (see later)18. In the past, however, studies of TLR4 have been problematic owing to LPS contamination in recombinant proteins, which might limit the informative value of some studies.

It has been postulated that crosstalk occurs between TLR4 and the complement system, both of which are involved in the initiation of the inflammatory response in sepsis19,20. Most strikingly, the complement anaphylatoxin C3a negatively regulates TLR4-mediated responses21. The extent of the regulatory effect of complement on TLR4-mediated cytokine production correlates with the level of complement-activation products and, in turn, the cytokines that are induced by TLR4 activation upregulate expression of the complement anaphylatoxin receptors C5AR and C3AR22,23. The finding that activation of TLR4 in platelets initiates the formation of neutrophil extracellular traps to ensnare bacteria in the vasculature further demonstrates the intricate interplay between innate immunity and the clotting system in sepsis24.

Owing to its prominent role in the initiation of the inflammatory response, TLR4 is a potential therapeutic target for sepsis. In a recent study, antibody-mediated blockade of TLR4 and MD2 protected against polymicrobial sepsis25 (TABLE 1). However, the mortality of septic mice that expressed a dysfunctional mutant TLR4 protein was not significantly different from that of wild-type mice with sepsis26. In human sepsis, clinical trials that blocked TLR4 did not show beneficial effects27, possibly because targeting TLR4 might only be an effective treatment for sepsis caused by Gram-negative bacteria or LPS, whereas the incidence of Gram-positive and fungal sepsis is increasing.

So, the early phase of sepsis, which is caused by the excessive activation of the host pathogen-recognition system by large-scale tissue damage and/or severe infection, leads to severe dysregulation of various body systems as a result of the pro-inflammatory environment.

**Harmful central hubs in sepsis**

The discovery that inflammatory mediators — not only invading microorganisms — are involved in the pathogenesis of sepsis has opened up a new avenue for the investigation of pathological mechanisms of inflammation. Many different mediators have been linked to the pathology of sepsis, some of which can be considered to be central hubs in the inflammatory
network (FIG. 1). Although they differ in terms of their source, kinetics of release and the stage of sepsis during which they predominate, these central hubs all have pleiotropic effects and connect various pathways of the immune response.

C5a. As part of the innate immune response, the complement system is activated during the early stages of sepsis, which generates large amounts of the anaphylatoxin C5a. At high concentrations, C5a has numerous harmful effects (reviewed in REF. 26; see later). Accordingly, C5a acts as a central mediator in sepsis by modulating other systems — including the coagulation cascade, TLR4-mediated responses and the release of cytokines, such as macrophage migration-inhibitory factor (MIF) and HMGB1 (REFS 10,19,27–29).

MIF. MIF, which was one of the first cytokines to be discovered, has a pivotal role in regulating systemic and local inflammatory responses (reviewed in REF. 50). Bacterial endo- and exotoxins, and pro-inflammatory mediators — such as tumour-necrosis factor (TNF), IFNγ and C5a — are strong inducers of MIF secretion by leukocytes28,38. Unlike other cytokines, MIF is constitutively expressed by leukocytes and stored intracellularly39. After its secretion, MIF functions as a classical pro-inflammatory cytokine and promotes innate and adaptive immune responses by activating macrophages and T cells39. Interestingly, the pro-inflammatory activities of MIF are mediated by its tautomerase activity, which is encoded by a domain containing an evolutionarily conserved catalytic site41. In addition to mediating its own pro-inflammatory effects, MIF also induces and amplifies the production of other pro-inflammatory cytokines and upregulates the expression of TLR4 by phagocytes39. At high concentrations, MIF prevents the p53-dependent apoptosis of activated macrophages, which results in sustained inflammatory responses40. However, the exact mechanisms by which MIF exerts its biological effects in the context of inflammation are not entirely clear. Although MIF activates intracellular signalling pathways after its endocytosis (which is an atypical mode of cytokine action)30, the CD74 receptor complex has also been described to function as a MIF receptor, from which signals are transduced through CD44 (REF. 32).

MIF is unique among cytokines in that it links the immune system with the endocrine system. In response to stress, MIF is secreted by the hypothalamus, the anterior pituitary gland and the adrenal glands30,33. Importantly, MIF antagonizes and overrides the anti-inflammatory effects of endogenous steroids36, which might have consequences for the well-established use of corticosteroids as a therapy for sepsis. Endogenous corticosteroids induce the release of MIF from immune cells, and so the inhibitory effect of MIF on the action of corticosteroids is a negative-feedback loop30,34,35. Excessive production of MIF is harmful in the acute phase of sepsis and plasma levels of MIF correlate with sepsis severity36. Neutralization of MIF or targeting of its tautomerase activity attenuated the inflammatory response and improved survival in experimental sepsis36,37. In addition, this treatment approach also markedly improved survival even when started after the onset of disease, which indicates that MIF is a promising therapeutic target36,37 (TABLE 1).

HMGB1. HMGB1 was originally described as a transcription factor34. After its redefinition as a pro-inflammatory cytokine39, HMGB1 became the focus of a large number of studies. HMGB1 is expressed by almost all cell types, except those lacking a nucleus

<table>
<thead>
<tr>
<th>System</th>
<th>Proposed mechanism</th>
<th>Target</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern-recognition system</td>
<td>Inhibition of PRRs to dampen the inflammatory response</td>
<td>TLR4 blockade</td>
<td>23</td>
</tr>
<tr>
<td>Pro-inflammatory mediators</td>
<td>Blockade of central hubs of the inflammatory response to reverse established sepsis</td>
<td>MIF blockade or inhibition of its tautomerase activity</td>
<td>36,37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMGB1 blockade</td>
<td>44,50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-17A blockade</td>
<td>52</td>
</tr>
<tr>
<td>Complement system</td>
<td>Neutralization of the harmful effects of C5a; formation of the MAC not affected</td>
<td>C5a neutralization</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dual C5AR and C5L2 blockade</td>
<td>29</td>
</tr>
<tr>
<td>Coagulation system</td>
<td>Induction of anticoagulant and anti-inflammatory effects</td>
<td>Administration of activated protein C</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Selective PAR1 and PAR2 activation</td>
<td>83</td>
</tr>
<tr>
<td>Autonomic nervous system</td>
<td>Activation of the cholinergic anti-inflammatory pathway and/or suppression of the adrenergic pro-inflammatory pathway to restore homeostasis</td>
<td>Parasympathetic branch: Pharmacological stimulation of α,γAChR on immune cells</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pharmacological stimulation of α,βAChR on immune cells</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vagus-nerve stimulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sympathetic branch: Pharmacological modulation of α- and β-adrenergic receptor pathways on leukocytes</td>
<td>12,108</td>
</tr>
</tbody>
</table>

α,γAChR, α- nicotinic acetylcholine receptor; C5AR, C5a receptor; C5L2, C5a-like receptor 2; HMGB1, high-mobility group box 1 protein; IL-17A, interleukin-17A; MAC, membrane-attack complex; MIF, macrophage migration-inhibitory factor; PAR1, protease-activated receptor 1; PRR, pattern-recognition receptor; RAGE, receptor for advanced glycation end-products; TLR4, Toll-like receptor 4.
The recent discovery of the IL-17A signal transduction is mediated by the vagus nerve of the autonomic nervous system. The signal transduction is mediated by TLR4 and acts as an endogenous alarmin to increase the release of pro-inflammatory mediators. TLR4-mediated responses, in turn, are negatively regulated by C5a. Similar to HMGB1, large amounts of MIF are released during sepsis, which promotes a pro-inflammatory response by amplifying cytokine secretion through the upregulation of TLR4 expression. MIF, which is produced by the pituitary gland as well as by leukocytes, inhibits the anti-inflammatory effects of endogenous glucocorticoids of the autonomic system, which, in turn, induce MIF secretion. HMGB1 links the immune response with the autonomic nervous system, which regulates the release of HMGB1 and other cytokines during sepsis. Interleukin-17A (IL-17A), which is an important regulator of inflammation at the interface between innate and adaptive immunity, orchestrates responses of both innate and adaptive immune cells.

Although HMGB1 is released systemically during sepsis, plasma levels do not necessarily correlate with outcome or survival. In contrast to other sepsis-associated cytokines, the peak of HMGB1 release occurs during later stages of the disease, and the levels of HMGB1 do not always decrease in patients who have recovered from sepsis. Pathogen-derived molecules and pro-inflammatory stimuli (such as TNF, IL-1β and IFNγ) induce the secretion of HMGB1 during inflammation. Interaction between C5a and its other receptor, C5a-like receptor 2 (C5L2), also triggers the release of HMGB1 in sepsis. Interestingly, HMGB1 secretion is under the influence of the ANS. Activation of the cholinergic anti-inflammatory pathway suppresses HMGB1 secretion by macrophages in sepsis and improves survival.

Owing to the pleiotropic effects of HMGB1 on the inflammatory response and its late release in sepsis, targeting HMGB1 might be a promising therapeutic strategy. In experimental settings, the direct blockade of HMGB1 or inhibition of RAGE improved survival in endotoxaemia and experimental sepsis (TABLE 1). Similar to the targeting of MIF, neutralization of HMGB1 prevented lethality when treatment occurred after the onset of sepsis and it reversed the development of multi-organ failure. However, the complexity of the underlying mechanisms of HMGB1 function precludes the use of HMGB1 blockade in clinical trials at this point.

**IL-17A**

The recent discovery of the IL-17 cytokine family, the members of which have emerged as important mediators of immune regulation, has greatly improved our understanding of the interplay between innate and adaptive immune responses (reviewed in REF. 51). IL-17A, the first described member of the IL-17 family, is a pro-inflammatory cytokine that is mainly produced by γδ T cells and IL-17A is also secreted by various other types of immune cell, including neutrophils, CD8+ T cells, natural killer cells, other Tγδ-cell subsets and γδ T cells. In brief, IL-17A is involved in mediating pro-inflammatory responses by triggering the production of many other cytokines (such as IL-1β, IL-6 and TNF) and provides crosstalk between lymphocytes and phagocytes.

It has recently been shown that increased IL-17A levels have adverse effects during experimental sepsis. Neutralization of IL-17A markedly improved survival, even when the treatment was administered as late as 12 hours after the initiation of experimental sepsis (TABLE 1). The protective effects of IL-17A blockade were associated with a marked attenuation of bacteraemia and markedly decreased plasma levels of pro-inflammatory cytokines. In agreement with these data, the *in vitro* production of pro-inflammatory mediators by macrophages in response to LPS was significantly increased in the presence of recombinant IL-17A. However, it is not yet known whether levels of IL-17A are increased in patients with sepsis, or during which phase of sepsis the neutralization of IL-17A would be beneficial in the clinical setting. Because the production of IL-17 is important for directing the immune response against some specific infections, the production of pro-inflammatory cytokines and chemokines is increased in patients with sepsis. HMGB1 links the immune response with the autonomic nervous system, the pituitary gland as well as by leukocytes, inhibits the anti-inflammatory effects of HMGB1 can be secreted by immune cells solely as a pro-inflammatory mediator, but might also function as a carrier or DAMP.
Endotoxaemia
This is caused by the presence of endotoxins, which are derived from Gram-negative bacteria, in the blood. It results in systemic activation of the inflammatory response, the development of shock and multi-organ failure and death. Models of endotoxaemia are used in experimental settings to induce systemic inflammation, but they do not necessarily mimic human sepsis.

Septic cardiomyopathy
The decreased myocardial function that occurs during sepsis-associated multi-organ failure. Hypotheses concerning the aetiology of this decreased function include impairment of mitochondrial function, dysfunction of the β-adrenoceptor-G protein-adenylate cyclase system, calcium-channel blockade by direct and indirect cardiodepressant factors and contractile impairment by activated leukocytes.

blockade of IL-17A under certain conditions might cause more harm than good. Therefore, it remains to be determined whether IL-17A is a useful target for therapeutic intervention in sepsis.

Dysregulation of plasmatic cascades
Complementopathy. The complement system can be activated through three different pathways, which converge on the generation of the anaphylatoxins C3a and C5a, C4a and the membrane-attack complex (MAC; also known as C5b–C9). In clinical studies of sepsis, increased concentrations of C3a, C4a and C5a in the plasma have been linked to poor outcome and survival. Interestingly, C3a might have anti-inflammatory properties in addition to acting as a pro-inflammatory anaphylatoxin. Mice with C3AR deficiency were more susceptible to endotoxin shock, which was accompanied by an increase in the concentration of pro-inflammatory cytokines in the plasma. Binding of C3a to C3AR can trigger the secretion of anti-inflammatory hormones by the pituitary gland, which might account for the anti-inflammatory properties of C3a.

New discoveries continue to increase our understanding of the numerous harmful effects of excessive C5a production during sepsis. The effects of C5a contribute to immunoparalysis, multi-organ failure, the apoptosis of thymocytes and adrenal medullary cells, and imbalances in the coagulation system (Fig. 2). In addition, C5a is involved in the development of septic cardiomyopathy. Decreased pressure in the left ventricle of the heart has been observed following sepsis, accompanied by defective contractility of cardiomyocytes; both of these effects were reversed by administration of a C5a-specific blocking antibody. Furthermore, when recombinant C5a was added to isolated rat cardiomyocytes in vitro, contractile dysfunction was induced, which indicates that excessive generation of C5a during sepsis causes cardiomyopathy.

Recent studies corroborate an important role for C5a in sepsis and indicate that it exerts its harmful effects in a complex manner. In addition to C5AR, C5a can bind specifically to a second receptor, C5L2, the function of which was unknown until recently. It was originally hypothesized that C5L2 functions as a decoy receptor for C5a, competing with C5AR for binding of C5a, although newer evidence indicates that C5L2 is a functional receptor. In human sepsis, the expression of C5L2 was downregulated on the surface of neutrophils during septic shock. The extent of this downregulation correlated with the development of multi-organ failure, which indicates that C5L2 contributes to the pathogenesis of sepsis. There is now evidence that C5AR and C5L2 cooperatively enhance the inflammatory response during sepsis, although each receptor might have specific and distinct functional roles. For example, C5a-induced release of MIF depends on C5AR signalling, whereas C5L2 mediates the C5a-dependent release of

Figure 2 | C5a is a central mediator of the inflammatory response in sepsis. During the early stages of sepsis, the complement system is systemically activated, generating large amounts of the anaphylatoxin C5a. C5a, which is a central molecule in the immunopathogenesis of sepsis, exerts its effects through interactions with its two receptors, C5a receptor (C5AR) and C5a-like receptor 2 (C5L2). The expression of these receptors is upregulated during sepsis, and their interactions with C5a contribute synergistically to harmful events in sepsis. The numerous effects of C5a include activation of the coagulation cascade by the induction of tissue-factor expression, which can result in disseminated intravascular coagulation (DIC). Furthermore, C5a triggers the release of pro-inflammatory cytokines, including macrophage migration-inhibitory factor (MIF) and high-mobility group box 1 protein (HMGB1), which contribute to the systemic inflammatory response syndrome (SIRS). In the later stages of sepsis, C5a is also responsible for sepsis-induced neutrophil dysfunction, leading to the shut down of intracellular signalling (immune paralysis) and increased susceptibility to secondary infections. C5a-induced apoptosis of thymocytes further aggravates immunosuppression, whereas the apoptosis of adrenal medullary cells results in insufficiency of the adrenergic system, eventually leading to septic shock. Recently, C5a and C5AR were also shown to be directly involved in the development of septic cardiomyopathy.
Disseminated intravascular coagulation

DIC, also known as consumptive coagulopathy, is a pathological process in which the blood begins to coagulate throughout the entire body. During this process, platelets and coagulation factors are depleted, resulting in a paradoxical situation in which there is a high risk of simultaneous fatal thrombosis and large-scale haemorrhage. DIC often occurs in critically ill patients with overwhelming infection, fulminant sepsis or malignancy.

Thrombin

Thrombin (also known as activated Factor II) is the central serine protease that converts soluble fibrinogen into insoluble strands of fibrin. It also catalyses many other coagulation-related reactions.

Tissue factor

A pro-coagulant factor that stimulates thrombus formation following contact with blood by accelerating the action of the coagulation factors Factor VIIa and Factor Xa. It can also be expressed on the surface of activated endothelial cells.

Activated protein C

A physiological anticoagulant. The activated form degrades Factor Va and Factor VIIIa of the coagulation cascade. The protein-C pathway has anti-thrombotic activity, as well as anti-inflammatory and anti-apoptotic functions. Administration of human recombinant activated protein C for the treatment of sepsis might block dysregulated coagulation, inhibit pro-inflammatory pathways and preserve organ function.

Thrombomodulin

An integral membrane protein that is expressed on the surface of endothelial cells. It functions as a co-factor in thrombin-induced activation of protein C in the anticoagulant pathway by forming complexes with thrombin. Thrombomodulin–thrombin complexes also stimulate fibrinolysis by cleaving thrombin-activatable fibrinolysis inhibitor (TAFI) into its active form.

HMGB1 from phagocytes. Importantly, blockade of either receptor protects against lethality in moderate forms of sepsis, but only the inhibition of both C5a receptors provides protection in severe sepsis.

Although inhibitors of other complement factors (such as a C1 inhibitor or soluble recombinant complement receptor 1) have had limited beneficial effects in the clinic, C5a might be a promising target for pharmaceutical intervention in sepsis. The advantage of this strategy is that the inhibition of the harmful effects of C5a does not interfere with the assembly of the MAC, which is essential for defence against invading microorganisms. Currently, dual blockade of C5AR and C5L2, rather than blockade of C5a alone, seems to be an encouraging strategy for clinical trials (TABLE 1).

Coagulopathy. In the clinical setting of sepsis, dysregulation of the coagulation cascade (BOX 1) results in major complications. The extent of activation of the coagulation cascade during sepsis can range from an insignificant level to the occurrence of disseminated intravascular coagulation (DIC). In the initial phase of DIC, thrombin activation results in intra- and extravascular fibrin formation (a process known as hypercoagulability), followed by the consumption of coagulation factors and platelet dysfunction (known as hypocoagulability). In the late phase of DIC, microvascular fibrin deposition is often associated with the development of multi-organ failure owing to perturbations in the microcirculation. As DIC develops, inflammation and coagulation interact in a bidirectional manner. Activated thrombin can promote the activation of various pro-inflammatory pathways — including the production of pro-inflammatory cytokines (such as TNF, IL-1β and IL-6) and the generation of C5a — and cytokines, in turn, can stimulate coagulation. Tissue factor, which is a central molecule in the initiation of DIC, is expressed by activated endothelial cells and by cells that are not normally exposed to blood flow, such as sub-endothelial cells and fibroblasts, and also by circulating immune cells. In sepsis, the pro-inflammatory environment causes mononuclear cells to upregulate the expression of tissue factor on their cell surface, leading to the systemic activation of coagulation.

Another consequence of DIC is the inhibition of fibrinolysis. In addition to endothelial-cell dysfunction during sepsis, which also occurs as a result of the pro-inflammatory environment, increased levels of plasminogen-activator inhibitor 1 (PAI1) and thrombin-activatable fibrinolysis inhibitor (TAFI) lead to impaired removal of fibrin. Also, the consumption of various factors that normally regulate the generation of thrombin, such as antithrombin III, protein C and tissue-factor pathway inhibitor (TFPI), contributes to the development of DIC.

Recombinant activated protein C is currently the only approved drug for the treatment of sepsis that targets the inflammatory response. Protein C, which is a regulator of the coagulation cascade, is activated by thrombin bound to thrombomodulin and by endothelial protein C receptor (EPCR) on endothelial-cell membranes. After dissociation from EPCR, activated protein C binds to its co-factor, protein S, which then results in the inactivation of clotting factors Va and VIIIa. In addition to its anticoagulant activity, activated protein C has profound anti-apoptotic and anti-inflammatory properties. It markedly decreases the apoptosis of endothelial cells and lymphocytes and exerts pro-fibrinolytic effects by inhibiting PAI1 (REFS 74,80). The anti-inflammatory effects of activated protein C are
mediated by EPCR and the cleavage of protease-activated receptor 1 (PAR1), which has a central role in linking coagulation and inflammation.\(^{61-63}\) (TABLE 1).

The protein-C pathway is particularly susceptible to inhibition by inflammatory responses in sepsis-associated DIC.\(^{71}\) In addition to a decrease in the level of protein C, the downregulation, shedding and cleavage of thrombomodulin and EPCR are the main causes of dysfunction of the protein-C pathway.\(^{64}\) HMGBl inhibits the protein-C pathway by interfering with the thrombin–thrombomodulin complex and it also promotes coagulation by stimulating tissue-factor expression and inhibiting tissue plasminogen activator (TPA), a serine protease on the surface of endothelial cells that activates plasmin of the fibrinolysis cascade.\(^{65}\)

The administration of activated protein C in sepsis suppresses pro-inflammatory cytokine production and decreases the adhesion of phagocytes to injured endothelium through EPCR- and PAR1-dependent signalling.\(^{66,67}\) However, the anticoagulant activity of activated protein C might exacerbate bleeding complications in patients who usually have a compromised clotting system.\(^{68}\) Future clinical trials should assess whether a form of activated protein C that does not have anticoagulant effects will improve clinical efficacy (that is, decrease sepsis mortality) and safety (that is, decrease the incidence of bleeding complications) in humans with sepsis.

**Linking complement and coagulation.** Traditionally, the complement and coagulation systems are described as separate cascades. As descendants of a common ancestral pathway, both are proteolytic cascades that are composed of serine proteases with common structural characteristics and similar activating stimuli.\(^{72,73}\) The relationship is not limited to the biochemical similarity in their serine proteases, however, as these two pathways are also linked by many mutual connections that form a complex network (FIG. 3).

During sepsis, the activated coagulation pathway predisposes to thrombosis and DIC, which further aggravate the excessive inflammatory response and complement activation.\(^{74}\) A well-known interaction between the complement and coagulation systems is the activation of the classical complement pathway by coagulation Factor XIIa, which can activate the complement component C1 [REF. 89]. More recently, it has been shown that thrombin can function as a C5 convertase in a C3-independent manner.\(^{80}\) This crosstalk is particularly interesting, not only because thrombin and C5a are central factors in their respective cascades, but also because this indicates that C5a and the MAC can be generated in the absence of upstream complement activation. Similar to thrombin, kallikrein and plasmin directly cleave C3 and its activation fragments.\(^{80,89}\) In an indirect negative-feedback loop, thrombin-activated TAFI inactivates C3a and C5a.

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**Figure 3 | Cross-talk between the complement, coagulation and fibrinolysis systems.** The complement system, the coagulation cascade and the fibrinolysis cascade communicate through many direct and bidirectional interactions (indicated by red arrows). Activated clotting Factor XII (FXIIa) can activate the classical complement pathway through cleavage of the complement component C1. Similarly, thrombin, kallikrein (not shown) and plasmin directly cleave complement component C3, as well as its activation fragments. Moreover, thrombin can cleave C5 into C5a, which occurs independently of C3 and therefore represents a bypass of the three traditional complement-activation pathways (that is, the classical, lectin and alternative pathways). Thrombin-activatable fibrinolysis inhibitor (TAFI) inactivates C3a and C5a in a negative-feedback loop. The complement system also amplifies coagulation through the C5a-mediated induction of expression of tissue factor and plasminogen-activator inhibitor 1 (PAI1) by leukocytes, the latter of which inhibits fibrinolysis. In addition, mannan-binding lectin serine protease 2 (MASP2) of the lectin complement-activation pathway triggers coagulation by converting prothrombin to thrombin. C4b-binding protein (C4BP) of the complement pathway inhibits protein S, which is a co-factor for the activated protein-C pathway of coagulation inhibition, which indicates that the inhibition of anticoagulant mechanisms further augments the pro-coagulant activities of complement, MAC, membrane-attack complex (C5b–C9); TPA, tissue plasminogen activator; UPA, urokinase-like plasminogen activator.
Box 2 | The autonomic nervous system

The autonomic nervous system (ANS) is part of the peripheral nervous system. It has three components: the parasympathetic branch, the sympathetic branch and the enteric nervous system. The ANS maintains homeostasis in the body by controlling vital functions that include heart rate, respiration rate, digestion, perspiration and body temperature. Traditionally, the sympathetic and parasympathetic branches of the ANS were thought to be endogenous neuronal antagonists. Therefore, the classical terminology referred to adrenergic responses (sympathetic) as ‘fight or flight’ and to cholinergic responses (parasympathetic) as ‘rest and digest’ responses. However, it is now clear that the relationship between these pathways is more complex.

Signal transmission in the parasympathetic branch of the ANS is mediated by acetylcholine and its receptors, which are abundantly expressed by many cell types. The main peripheral component of the parasympathetic branch of the ANS is the vagus nerve.

The sympathetic branch of the ANS consists of sympathetic neurons and the adrenal medulla. Catecholamines, which are the main mediators of the sympathetic branch, mediate pleiotropic effects by interacting with adrenergic receptors that are ubiquitously expressed by nearly all tissue and cell types. The activation of adrenergic receptors triggers an intricate intracellular signalling network that has yet to be fully understood.

The enteric nervous system, which controls the gastrointestinal system, is also considered to be part of the ANS. Anatomically, the enteric nervous system consists of a large number of neurons that are embedded in the lining of the gastrointestinal tract. Although the enteric nervous system can operate autonomously, it communicates closely with the central nervous system, and is associated with a considerable amount of sympathetic and parasympathetic innervation.

The complement system amplifies coagulation by modification of phospholipid membranes (which is required for the initiation of coagulation through tissue factor), by activating platelets and by inducing the expression of tissue factor and PAI1 by leukocytes. Accordingly, blockade of C5a during experimental sepsis markedly ameliorated the effects of DIC. In addition, mannan-binding lectin serine protease 2 (MASP2), a protease that is characteristic of the lectin pathway of complement activation, can activate coagulation by cleaving prothrombin into active thrombin. The pro-coagulant activities of complement are increased when anticoagulant mechanisms are inhibited; for example, the formation of a complex between C4b-binding protein and protein S results in a decrease in the availability of protein S to act as a co-factor for the anticoagulant protein-C pathway. In addition, several indirect influences of the complement system on coagulation are mediated through other pro-inflammatory factors (such as TNF, IL-6 and HMGB1) have been documented.

In summary, the complement, coagulation and fibrinolytic systems are tightly connected through multiple direct interactions of serine proteases, which together make up a ‘plasma serine-protease network’. In the setting of sepsis, crosstalk between the complement and coagulation pathways is of particular importance, as their uncontrolled activation is an essential contributor to the pathogenesis of the disease.

The parasympathetic ANS. Signalling of the vagus nerve by engagement of cholinergic receptors expressed by phagocytes has an important regulatory role in inflammation. Activation of αnACHRs, either by vagus-nerve stimulation or by αnACHR agonists, decreases intracellular cytokine synthesis by macrophages and dampens the inflammatory response. In other words, inflammation is under neuronal control by the ANS, which can reflexively modulate the inflammatory response by inhibiting the production of pro-inflammatory cytokines. This concept has therefore been termed the ‘inflammatory reflex’. The efferent arm of the inflammatory reflex is the cholinergic anti-inflammatory pathway, which is a robust regulator of cytokine production.

Recent studies have shown that the branch of the vagus nerve that innervates the spleen is crucial for the suppression of cytokine synthesis in sepsis. The spleen is an important source of TNF during sepsis and splenectomy significantly decreases systemic and hepatic levels of TNF in septic mice. In addition, direct cholinergic modulation of immune cells that transit through the spleen contributes to the regulation of inflammation at distant sites. In experimental sepsis, activation of the cholinergic anti-inflammatory pathway inhibited the production of pro-inflammatory mediators (including HMGB1 and TNF) and markedly increased survival, even when carried out as late as 24 hours after the onset of disease.

The sympathetic ANS. The main transmitters of the sympathetic branch of the ANS are catecholamines, which act through binding to adrenergic receptors. The early phase of sepsis is characterized by high concentrations of circulating catecholamines, which boost the initial inflammatory response. Later in septic shock, the production and release of endogenous catecholamines can become insufficient for maintaining equilibrium of the cardiovascular system (as indicated by the need for catecholamine administration during septic shock). The depletion of endogenous catecholamine sources might be caused by the apoptosis of adrenal medullary cells.
The concomitant dysfunctional adrenergic modulation of heart and blood vessels during septic shock indicates that impairment of adrenergic regulation contributes to cardio-circulatory failure.\textsuperscript{105}

It was originally thought that the synthesis of catecholamines was carried out only by the neuronal cells of the sympathetic branch of the ANS, but it has now been shown that leukocytes are also an abundant source of catecholamines.\textsuperscript{12,99} Leukocytes also express adrenergic receptors, which indicates that catecholamines might have autocrine and/or paracrine effects on immune cells.\textsuperscript{12,99} The activation of adrenergic receptors on immune cells triggers distinct and finely tuned cytokine responses through NF-κB-dependent mechanisms.\textsuperscript{12,99}

During sepsis, catecholamines exert immunomodulatory effects through α- and β-adrenergic receptors that are expressed by immune cells.\textsuperscript{106–108} Stimulation of these receptors alters lymphocyte trafficking, vascular perfusion and cell proliferation and apoptosis, thereby affecting the functional responses of leukocytes.\textsuperscript{109–111} The response of neutrophils and macrophages in particular underlies adrenergic regulation by catecholamines, as the release of pro-inflammatory cytokines by these cells is tightly regulated by α-adrenergic receptors.\textsuperscript{112} Catecholamines might also contribute to the deleterious effects of sepsis through direct stimulation of bacterial growth in the gastrointestinal system, which might contribute to bacteremia through the translocation of enteric bacteria into the lymphatic and blood compartments.\textsuperscript{112}

In summary, activation of the adrenergic pathways of the sympathetic branch of the ANS during the early phase of sepsis promotes pro-inflammatory responses and aggravates adverse events, although the mechanisms that underlie these effects have yet to be evaluated in detail.

The enteric nervous system. Sepsis can occur as a result of inflammation in the abdominal cavity (known as peritonitis) that results from the disruption of barriers that protect the sterile compartment of the abdominal cavity from pathogens in the intestinal lumen. It was hypothesized that the translocation of enteric bacteria into the blood might also occur when the gut was not the main source of inflammation (for example, in situations such as pneumonia or burn injury), due to a general loss of intestinal epithelial barrier functions as a result of the pro-inflammatory environment. Recent research has shown that the gut can produce large amounts of catecholamines during sepsis, which are released into intestinal blood.\textsuperscript{113} Catecholamines that drain from the intestines through the portal vein into the liver can alter the functional state of Kupffer cells and hepatocytes through α2-adrenergic receptor signalling,\textsuperscript{104} ultimately contributing to the release of pro-inflammatory cytokines, hepatocellular dysfunction and liver failure.\textsuperscript{113,115} Catecholamine-induced activation of Kupffer cells might also be an important source of the cytokine storm during sepsis.\textsuperscript{116} Additional studies are required to identify the cell source of intestine-derived catecholamines during sepsis. It remains possible that either the enteric nervous system or resident immune cells in Peyers patches and lymph nodes of the intestinal system (or both) are responsible for the generation of intestine-derived catecholamines during sepsis.

ANS-targeted therapy. Modulation of the ANS might be a promising approach for the treatment of sepsis as an alternative to blocking pro-inflammatory mediators directly (TABLE 1). Whereas the cholinergic pathway attenuates the immune response and is considered to be anti-inflammatory,\textsuperscript{12,99} adrenergic stimulation promotes the release of pro-inflammatory cytokines and the recruitment of leukocytes (FIG. 4). Given that an imbalance between these two branches of the ANS contributes to the development of sepsis, stimulation of the cholinergic vagus nerve and/or suppression of adrenergic pathways might help to restore homeostasis. It has recently been shown that transcutaneous electrical stimulation of the vagus nerve improved survival and decreased the level of pro-inflammatory mediators in experimental sepsis.\textsuperscript{104} Although this approach might not yet be ready for use in the clinic, the fact that this treatment for sepsis would be non-invasive and independent of pharmacokinetics — unlike the administration of drugs — makes it particularly attractive.
**Concluding remarks**

Despite more than 20 years of extensive research, none of the promising therapeutic approaches for sepsis that target the inflammatory response has been successfully translated to the clinical setting, and rates of sepsis mortality have not decreased\(^1\). These failures are in part due to the fact that some of the animal models for sepsis that are used, such as endotoxaemia, do not accurately mimic sepsis in humans and/or because the limitations of animal models have been disregarded (reviewed in REF 118). In addition, it is now clear that the belief that a single key mediator causes sepsis, and that neutralization of such a factor could be a cure for all patients with sepsis, is erroneous. Instead, we now understand that sepsis is a complex, dynamic syndrome with great heterogeneity, and not a distinct disease. Sepsis can result from various causative insults, and susceptibility can be influenced by premorbid factors that include ethnicity, gender, age, genetic defects and environmental factors\(^19\). In particular, genetic and epigenetic changes, such as mutations in genes that encode PRRs or mediators of inflammation and their receptors, might have consequences for the host response. Because the underlying inflammatory response during sepsis varies between individual patients, various options for therapy should be made available. Ideally, individual patients should be precisely monitored for changes in characteristic markers of the host immune response to aid in the choice of specific immunomodulatory therapies.

We now know that the underlying inflammatory response in sepsis involves a complex interplay of different biological systems and cell types, resulting in severe dysregulation of the inflammatory network (FIG. 5). We are just beginning to understand the underlying regulatory pathways of this network. The application of interdisciplinary approaches will improve our knowledge of the molecular biology of inflammation in the context of sepsis. Initial large-scale programs that aim to uncover the molecular mechanisms of inflammation — which include the fields of surgery, genomics, proteomics, biostatistics, bioinformatics, computational biology and genetics — are now underway\(^20\). Although our current knowledge regarding inflammation might be in its infancy, recent progress indicates that patients will one day benefit from more advanced knowledge of the inflammatory response in sepsis.

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**Figure 5 | The inflammatory network in sepsis.** During sepsis, homeostasis between the various biological systems of the inflammatory network is highly imbalanced. In the initiation of sepsis, the release of a large amount of damage-associated molecular patterns (DAMPs) from invading microorganisms and/or damaged host tissue results in the overstimulation of pattern-recognition receptors (PRRs) on immune cells. Activated immune cells release excessive amounts of pro-inflammatory mediators (resulting in a ‘cytokine storm’), free radicals and enzymes, which converts the normally beneficial effects of inflammation into an excessive response that damages the host. Activation of the adrenergic branch of the autonomic nervous system (ANS) and/or decreased activity of the cholinergic anti-inflammatory pathway (of the parasympathetic branch of the ANS) further amplifies the pro-inflammatory responses of neutrophils, macrophages and dendritic cells in sepsis. The presence of invading microorganisms or their products in the blood can cause systemic activation of the complement system, which results in the excessive generation of complement anaphylatoxins, which, at high concentrations, induce numerous harmful effects. Simultaneous activation of the coagulation system and the inhibition of fibrinolysis as a result of the pro-inflammatory environment and/or damaged endothelium can result in disseminated intravascular coagulation (DIC), which is a major complication of sepsis, and in the amplification of the inflammatory response. The complement, coagulation and fibrinolysis systems are tightly connected through direct interactions of serine proteases, and imbalances in each cascade are intensified in a positive-feedback loop (FIG. 4). Finally, the sustained pro-inflammatory environment affects the functional state of immune effector cells, eventually causing the dysfunction of neutrophils and immunosuppression. Alterations in leukocyte apoptosis in the later stages of sepsis further account for immunosuppression, which increases the susceptibility to secondary infections.

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11. This study shows that thrombin can act as a C5 convertase, which indicates that there is direct crosstalk between the coagulation and complement systems.
16. In this study, TLR4 is defined as the recognition receptor for LPS.
This work defined, for the first time, an extracellular role for HMGB1 in the inflammatory mediator in endotoxaemia and sepsis.


This publication is the most recent study to describe the epidemiology of sepsis in the United States. Most importantly, the rates of hospitalization and mortality from severe sepsis increased significantly over the observation period from 1993 to 2003.