**ABSTRACT**

Sepsis is a very heterogeneous clinical syndrome broadly defined as the systemic host response to an infection. Until very recently, the prevailing concept of the pathogenesis of sepsis was that mortality is the consequence of an uncontrolled hyperinflammatory response of the host. The disappointing results of nearly 40 years of anti-inflammatory strategies and the development of animal models that more closely mimic clinical sepsis have led to the reconsideration of the pathophysiology of sepsis. Sepsis is now considered a misbalance between proinflammatory reactions (designed to kill invading pathogens but at the same time responsible for tissue damage) and anti-inflammatory responses (designed to limit excessive inflammation, but at the same time making the host more vulnerable for secondary infections). This review discusses key components of the pro- and anti-inflammatory response to sepsis, listing potential novel interventional strategies along the way.

**KEYWORDS**

Cytokines, coagulation, innate immunity, sepsis

**HISTORICAL PERSPECTIVE**

The original theory that sepsis mortality is caused by an excessive stimulation of the immune system by high bacterial loads was based on studies in animals that were infused with large doses of bacteria or bacterial products, in particular lipopolysaccharide (LPS), the toxic component of the Gram-negative bacterial cell wall. Such infusions result in a strong activation of different proinflammatory protein cascades which, although designed to protect the host against invading pathogens, can cause damage to tissues when produced in high amounts. In a hallmark article published in 1985, Beutler and colleagues reported that neutralisation of a single proinflammatory cytokine – tumour necrosis factor (TNF)α – secreted after intravenous injection of an otherwise lethal dose of LPS prevented death in mice.1 Two years later, these results were confirmed by Tracey and colleagues, who showed that a monoclonal anti-TNFα antibody protected baboons against lethal Gram-negative sepsis elicited by intravenous infusion of high quantities of viable *Escherichia coli*.2 Since then anti-TNFα interventions have been reported to protect against lethality in a number of sepsis models in which high doses of bacteria or bacterial products were administered systemically.3 In addition, elimination of another proinflammatory cytokine, interleukin (IL)-1, also reduced lethality induced by LPS or living bacteria in animals.4,5 These early experimental sepsis studies resulted in the design and performance of many clinical trials seeking to inhibit either TNFα or IL-1 activity in patients with severe sepsis. Unfortunately, virtually all clinical sepsis trials with anti-TNFα strategies and recombinant IL-1 receptor antagonist failed, and many other anti-inflammatory strategies were also not successful in altering the outcome of patients with sepsis. As such, the hypothesis that excessive inflammation is the main or sole underlying cause for an adverse outcome of a septic patient is not correct.

**INDUCTION OF AN INNATE IMMUNE RESPONSE TO BACTERIA**

The innate immune system is able to detect pathogens via a limited number of pattern-recognition receptors (PRRs).6,7 PRRs recognise conserved motifs expressed by pathogens, known as pathogen-associated molecular patterns (PAMPs).
Examples of bacterial PAMPs are LPS, expressed by all virulent Gram-negative bacteria, peptidoglycan, lipopeptides (constituents of many pathogens), lipoteichoic acid (a cell wall component of Gram-positive bacteria), flagellin (factor in the mobility of bacteria) and bacterial DNA.\textsuperscript{6,7} Additionally, PRRs can also recognise endogenous mediators released upon injury, thereby warning the host for imminent danger. Such endogenous danger signals have been named ‘alarmins’ or ‘danger-associated molecular patterns’ (DAMPs). Heat shock proteins, fibrinogen, hyaluronic acid and high-mobility group box-1 protein (HMGB-1) are examples of DAMPs that cause further amplification of the proinflammatory response through Toll-like receptor (TLR) 4 (see below).

A specific family of PRRs named Toll-like receptors (TLRs) play a pivotal role in the initiation of cellular innate immune responses. Thirteen TLRs (TLRs 1 to 13) have been identified in mammals. Bacterial ligands for most TLRs have been described; table 1 summarises TLR specificity for several bacterial PAMPs with probable relevance for sepsis. The entire TLR family signals via four adapter proteins (myeloid differentiation primary-response protein 88 [MyD88], TIR domain-containing adaptor protein (TIRAP), TIR domain-containing adaptor protein-inducing IFN\(\beta\) [TRIF] and TRIF-related adaptor molecule [TRAM]), which together with a number of protein kinases take care of the recognition and response to microbial molecules. With regard to the role of TLRs in sepsis, it should be noted that TLRs are on the one hand essential for the early detection of pathogens, but on the other hand may also cause excessive inflammation after uncontrolled stimulation.\textsuperscript{7} As an example, TLR4 deficient mice are fully protected against LPS-induced lethality, but these animals display an enhanced susceptibility to several Gram-negative infections.\textsuperscript{9} The clinical relevance of TLR signalling is reflected by the recent description in children with a genetic deficiency for MyD88 or IL-1 receptor-associated kinase 4 (IRAK4), a kinase acting directly downstream from MyD88, who are especially vulnerable to purulent infections.\textsuperscript{9,10} In addition, several single nucleotide polymorphisms in genes encoding TLRs have been associated with an altered susceptibility to bacterial infections.\textsuperscript{11}

Several other innate immune receptors have been implicated in the recognition of bacteria and induction of a host inflammatory response after infection. Whereas TLRs detect pathogens at either the cell surface or in lysosomes/endosomes, microorganisms that invade the cytosol can be recognised by cytoplasmatic PRRs, among which nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs).\textsuperscript{12} Several members of the NLR family can assemble multimolecular complexes termed ‘inflammasomes’ in response to various activators, leading to the activation of inflammatory caspases. Activation of the NLRP3 inflammasome by PAMPs or DAMPs induces activation of caspase-1, which causes the processing of the proinflammatory cytokines interleukin (IL)-1\(\beta\) and IL-18.\textsuperscript{12} Although it has become clear that NLRs are of utmost importance for the recognition of bacteria by the innate immune system, their exact role in sepsis pathophysiology is far from clear.

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**Table 1. Pathogen and danger associated molecular patterns and their recognition by Toll-like receptors**

<table>
<thead>
<tr>
<th>Pathogen-associated molecular patterns</th>
<th>Species</th>
<th>TLR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>Gram-negative bacteria</td>
<td>TLR4</td>
</tr>
<tr>
<td>Lipoteichoic acid</td>
<td>Gram-positive bacteria</td>
<td>TLR2*</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Most bacteria</td>
<td>TLR2</td>
</tr>
<tr>
<td>Triacyl lipopeptides</td>
<td>Most bacteria</td>
<td>TLR1/TLR2</td>
</tr>
<tr>
<td>Diacyl lipopeptides</td>
<td>Mycoplasma spp</td>
<td>TLR2/TLR6</td>
</tr>
<tr>
<td><strong>Porins</strong></td>
<td>Neisseria</td>
<td>TLR2</td>
</tr>
<tr>
<td>Flagellin</td>
<td>Flagellated bacteria</td>
<td>TLR5</td>
</tr>
<tr>
<td>CpG DNA</td>
<td>Bacteria</td>
<td>TLR9</td>
</tr>
<tr>
<td>Unknown</td>
<td>Uropathogenic bacteria</td>
<td>TLR1‡</td>
</tr>
<tr>
<td><strong>Danger-associated molecular patterns</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat shock proteins</td>
<td>Host</td>
<td>TLR4</td>
</tr>
<tr>
<td>Fibrinogen, fibronecrtin</td>
<td>Host</td>
<td>TLR4</td>
</tr>
<tr>
<td>Hyaluronan</td>
<td>Host</td>
<td>TLR4</td>
</tr>
<tr>
<td>Biglycans</td>
<td>Host</td>
<td>TLR4</td>
</tr>
<tr>
<td>HMGB1</td>
<td>Host</td>
<td>TLR4, TLR2</td>
</tr>
</tbody>
</table>

The table shows PAMPs and DAMPs with likely relevance for bacterial sepsis (PAMPs expressed by fungi, viruses and parasites are not shown). *For detection of LTα from some pathogens TLR6 functions as a coreceptor for TLR2. ‡TLR11 is not functional in humans. **Recent studies describe a role for TLRs in acute injury using rodent models of haemorrhagic shock, ischaemia and reperfusion, tissue trauma and wound repair, and various toxic exposures; these studies have implicated TLR4 as a major factor in the initial injury response. The table shows endogenous mediators identified as TLR4 ligands.
HMGB1 and RAGE

HMGB1, a nuclear protein that stabilises nucleosome formation, has been implicated in the pathogenesis of sepsis. Patients with sepsis demonstrate elevated circulating levels of HMGB1. LPS-induced shock in mice was associated with a relatively late release of HMGB1 into the circulation; importantly, an anti-HMGB1 antibody protected against LPS-induced lethality even when the administration was postponed until after the peak levels of TNFα and IL-1 had been reached. Delayed administration of anti-HMGB1 also improved survival in a model of abdominal sepsis. Considering that the therapeutic window for anti-HMGB1 therapies is much wider than for TNF-neutralising strategies, inhibitors of HMGB1 may be valuable as an adjunctive therapy for severe sepsis.

It is uncertain whether highly purified HMGB1 can directly activate cells. It has been suggested that other molecules bound by HMGB1 are at least in part responsible for this. Nonetheless, several receptors have been implicated in mediating the cellular effects of HMGB1, including TLR2 and TLR4, and the receptor for advanced glycation end products (RAGE). RAGE is a promiscuous receptor that interacts with diverse ligands such as advanced glycation end products, S100/calgranulins, amyloid A, leucocyte adhesion receptors, Escherichia coli curli operons and HMGB1. The potential role of RAGE signalling in sepsis pathophysiology has been documented in mice with abdominal sepsis: both RAGE-deficient mice and wild-type mice treated with soluble RAGE were partially protected against lethality in this model of severe sepsis. In addition, RAGE-deficient mice demonstrated an improved host defence during pneumococcal pneumonia. Further research is warranted to address the therapeutic potential of RAGE (ligand) inhibitors in sepsis.

Macrophage migration inhibitory factor (MIF) is a cytokine that can be produced by many different cell types. Serum MIF levels are elevated in patients with sepsis. MIF regulates innate immune responses through modulation of TLR4: when MIF-deficient mice were challenged with LPS they showed a defective response as a direct result of decreased TLR4 expression. Inhibition of MIF activity with neutralising anti-MIF antibodies protected mice from septic shock. These data suggest that MIF-directed therapies offer a new treatment opportunity for sepsis. Intriguingly, however, polymorphisms associated with higher MIF expression were recently shown to be associated with a reduced 90-day mortality in patients with community-acquired pneumonia. These new data prompt caution in the clinical application of anti-MIF strategies in infectious diseases.

Myeloid-related protein 8 and Mrp14

Myeloid-related protein 8 (Mrp8 also called S100A8) and Mrp14 (also called S100A9) are members of the S100 protein family. Mrp8 and Mrp14 can form heterodimers that elicit a variety of inflammatory responses. Mrp8/14 complexes can activate TLR4 and amplify the LPS-triggered inflammatory responses of phagocytes. In patients with sepsis and in healthy humans injected with LPS elevated Mrp8/14 plasma levels have been observed. Mice lacking Mrp8-Mrp14 complexes had an increased survival during LPS-induced lethal shock and bacterial sepsis, and displayed a reduced bacterial dissemination after intraperitoneal infection with E. coli. It remains to be established whether inhibition of Mrp8/14 could be a useful adjunctive therapy for clinical sepsis.

C5a and C5a receptor

Although the complement system has traditionally been considered a central part of host defence against invading pathogens, complement activation may also contribute to an adverse outcome of sepsis. Indeed, infusion of anti-C5a antibodies improved haemodynamic parameters in pigs infused with LPS or live E. coli and reduced mortality in primates with E. coli sepsis and rats subjected to coecal ligation and puncture. As such, interventions seeking to block C5a signalling represent promising targets for sepsis treatment, although as with other anti-inflammatory strategies, an important goal of complement inhibition would be to avoid disrupting the role of complement in host defence.
Patients with sepsis almost invariably show evidence for activation of the coagulation system. Tissue factor (TF) is regarded as the primary initiator of coagulation in sepsis. The pivotal role of TF in activation of coagulation during endotoxaemia and sepsis has been established by many different experiments. In particular, a number of different strategies that prevent the activation of the TF pathway in endotoxaemic humans and chimpanzees, and in bacteraemic baboons abrogated the activation of the common pathway of coagulation, which in septic baboons was accompanied by a reduced mortality. Procoagulant events are controlled by three major anticoagulant proteins: tissue factor pathway inhibitor (TFPI), antithrombin and activated protein C (APC). During severe sepsis the activities of TFPI, antithrombin and the protein C-APC system are impaired, which together with enhanced TF-dependent coagulation results in a shift toward a net procoagulant state. In septic primates the administration of either TFPI, antithrombin or APC attenuated consumptive coagulopathy. Large phase III clinical trials in sepsis patients have been completed with these three anticoagulants. Only recombinant human APC was found to reduce 28-day mortality in patients with severe sepsis; importantly, APC was not effective in patients with severe sepsis and a low risk of death. Recently, the European licensing authorities have requested Eli Lilly (the manufacturer of recombinant human APC) to perform another placebo-controlled trial with APC in adult patients with severe sepsis; this trial (PROWESS-SHOCK) was recently initiated.

In recent years, much attention has been given to the role of protease-activated cell receptors (PARs) in linking coagulation and inflammation. The PAR family consists of four members, PAR-1 to PAR-4, which are localised in the vasculature on endothelial cells, mononuclear cells, platelets, fibroblasts and smooth muscle cells. Recently, cell penetrating peptides (so-called pepducins) were used to delineate the roles of PAR-1 and PAR-2 in LPS shock and abdominal sepsis. Evidence was provided that activation of PAR-1 is harmful during the early phases of endotoxaemia and sepsis, facilitating pulmonary leak and disseminated intravascular coagulation, but becomes beneficial at later stages. Remarkably, PAR-1 deficiency was reported to protect mice against LPS-induced lethality in an LD80 model of endotoxaemia at least in part through interruption of the PAR-1 mediated amplification of systemic inflammation. More studies are warranted to determine the potential value of PAR signalling inhibitors for the treatment of sepsis.

Although severe infection may be associated with an early phase of hyperinflammation, in most if not all patients who survive the acute phase of sepsis, a prolonged state of immune suppression evolves, a condition referred to as immunoparalysis. Indeed, the greater part of patients who are enrolled in sepsis trials display evidence of this state of reduced immune responsiveness: their blood leucocytes are less capable of releasing proinflammatory cytokines upon stimulation with bacteria or bacterial products. Although immunoparalysis has been regarded as beneficial in the sense that it counteracts a potential devastating pro-inflammatory response, it can also lead to an inability to clear infection and a subsequent predisposition to nosocomial infection. Experimental data have provided firm evidence for a causal role for enhanced apoptosis in the pathogenesis of sepsis, i.e. prevention of apoptosis of lymphocytes or the intestinal epithelium improved survival in experimental sepsis.

The cholinergic nervous system, and in particular the vagus nerve, represents another host response pathway designed to limit inflammatory responses. In the so-called cholinergic anti-inflammatory pathway enhanced efferent activity of parasympathetic nerve endings results in the release of acetylcholine, which by a specific action on 7 cholinergic receptors on macrophages suppresses proinflammatory cytokine production. Disruption of this neural-based system by vagotomy renders animals more vulnerable to LPS toxicity. Conversely, electrical stimulation of the efferent vagus nerve prevented the development of shock and attenuated the release of TNFα, whereas stimulation of 7 cholinergic receptors by specific agonists, such as nicotine, attenuated systemic inflammation and improved the outcome of mice with polymicrobial abdominal sepsis. Together, these preclinical data suggest that stimulation of the vagus nerve and/or pharmacological 7 cholinergic receptor agonists may be a useful strategy in the treatment of the severe inflammation accompanying sepsis.

Conclusion

Sepsis can be defined as the host response to infection. For many years this response was considered to be dictated by an overwhelming inflammatory reaction to invading bacteria. Although some septic patients may succumb from the initial exacerbated hyperinflammatory

COAGULATION AND ANTICOAGULATION

IMMUNE SUPPRESSION AND APOPTOSIS

THE CHOLINERGIC ANTINFLAMMATORY PATHWAY

CONCLUSION
response, the majority of patients die during the following extended period of immunodepression. A careful balance between the inflammatory and anti-inflammatory response is vital for a successful host response to sepsis. Intervening in this delicate balance in order to improve sepsis outcome will be a major challenge for the years to come.

**ACKNOWLEDGMENT**

W.J. Wiersinga is supported by a VENI grant from the Netherlands Organisation of Scientific Research.

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