

## W Host-pathogen interactions in sepsis

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Sepsis is a major health problem. The concept that sepsis mortality is the result of an uncontrolled hyperinflammatory host response has recently been challenged. It is now widely thought that the host response to sepsis involves many, concomitant, integrated, and often antagonistic processes that involve both exaggerated inflammation and immune suppression. Several novel mediators and pathways have been shown to play a part. Moreover, evidence is accumulating that microbial virulence and bacterial load contribute to the host response and the outcome of severe infections. A complex and dynamic interaction exists between pathogens and host immune-defence mechanisms during the course of invasive infection. Some pathogens have acquired the capacity to communicate with each other and sense the host's vulnerabilities. Bidirectional signals are detectable at the critical interface between the host and microbial invaders. The outcome of this interaction determines the fate of the host at the outset of the septic process. A formidable array of innate and acquired immune defences must be breached if a pathogen is to successfully disseminate and cause severe sepsis and septic shock. This Review summarises current knowledge of microbial pathogenesis and host–pathogen interactions during sepsis and the ensuing development of potential therapeutics.

### Introduction

Sepsis is the second most common cause of death in non-coronary intensive care units and the tenth leading cause of death overall in high-income countries.<sup>1,2</sup> During the past two decades, the incidence of sepsis has increased annually by 9% to reach 240 per 100 000 population in the USA by 2000.<sup>3</sup> Until very recently, the prevailing concept of the pathogenesis of sepsis was that mortality is the consequence of an uncontrolled hyperinflammatory, predominantly cytokine-mediated, response of the host. In part because of the failure of dozens of clinical trials that assessed anti-inflammatory agents in severe sepsis, and in part because of growing insights from preclinical models that more closely resemble clinical sepsis than originally used in this area of research, current knowledge of host–pathogen interactions and their consequences in sepsis have increased tremendously. Additionally, virulence and bacterial load are now thought to contribute to the host response and the outcome of severe infections. This Review summarises recent advances in the understanding of microbial pathogenesis and host–pathogen interactions during severe sepsis. The increased insights into the pathogenesis of sepsis have led to the design and development of novel therapies, some of which have reached the clinical phase of assessment.

### The pathogen: microbial pathogenesis and virulence characteristics

#### Causative microorganisms

Whereas, until the early 1980s, Gram-negative bacteria were the predominant organisms that caused sepsis, the incidence of Gram-positive sepsis has steadily increased. In a large survey done in 2000 in the USA, Gram-positive bacteria accounted for 52.1% of sepsis cases, Gram-negative bacteria 37.6%, polymicrobial infections 4.7%, anaerobes 1.0%, and fungi 4.6%; the greatest relative changes were seen in the incidence of Gram-positive and fungal infections.<sup>3</sup> The increasing frequency of fungal sepsis is a worrisome trend because

this form of sepsis has a particularly poor prognosis. The most commonly isolated Gram-positive bacterial pathogens are *Staphylococcus aureus* and *Streptococcus pneumoniae*, and the most common Gram-negative pathogens are *Escherichia coli*, *Klebsiella* spp, and *Pseudomonas aeruginosa*.<sup>4</sup>

#### Expression and regulation of microbial virulence

Microbial genomics have established the remarkable array of genetic determinants that are needed for the full expression of microbial virulence.<sup>5</sup> Pathogenic strains of bacterial species differ from commensal strains by the acquisition and expression of specific clusters of virulence genes. Potential pathogens face enormous challenges when attempting to invade a human host. They must attach to host tissue, cross the mucosal surface or integument, replicate, and disseminate faster than the host's antimicrobial defence systems.<sup>6,7</sup>

A myriad of rather ingenious defensive and offensive weaponry are expressed by microbial invaders in sepsis.<sup>8</sup> Global regulators of the entire collection of virulence genes (known as the virulome) have recently been characterised.<sup>9</sup> Virulence genes scattered across the bacterial chromosome are now recognised to work together in patterns with sequential sets of transcriptional programmes. The regulation of virulence expression is increasingly being elucidated, and this may offer new therapeutic targets in the care of septic patients.<sup>6</sup>

#### Bacterial toxins

Much of the damage inflicted on the septic host is attributable to microbial toxins and the host's response to them. There are many extracellular enzymes and microbial mediators that contribute to tissue injury in sepsis. Three functional classes of toxins exist and three basic delivery systems are used by bacterial pathogens. Type I toxins cause injury to the host without entering host cells. Superantigen-mediated toxic shock syndrome produced by either *S aureus* or *Streptococcus pyogenes* exemplify these toxins.<sup>10</sup> Type II toxins are direct

eukaryotic membrane toxins and include haemolysins and phospholipases produced by various microbial pathogens. These toxins damage cell membranes of host cells and allow pathogens access to intracellular contents while disrupting the host cellular response to invading pathogens at the onset of sepsis. Type III toxins are known as A/B toxins owing to their obligate binary nature. The specific binding moiety (the B component) links with an active enzymatic component (the A moiety). Many well-known bacterial toxins, such as cholera toxin, anthrax lethal toxin, and shiga-like toxin, are examples of type III toxins. Many common human pathogens, such as *S aureus*, *S pneumoniae*, *S pyogenes*, *E coli*, and *P aeruginosa*, secrete an array of A/B toxins during microbial invasion. These toxins work in concert to damage cellular defences, break down barriers to invasion, and allow the pathogen to disseminate within the host.

Bacterial exotoxins are secreted by various mechanisms of which the type III secretion system is perhaps the most ingenious. Type III secretion systems emanate from a clustered set of linked genes that include over 20 gene products. This system has a sensing mechanism that detects the cell surface of host cells. A needle-like projection system is then assembled whereupon an array of intracellular toxins are delivered directly into the cytoplasm in target cells.<sup>11</sup>

One crucially important microbial toxin in the pathogenesis of sepsis is lipopolysaccharide. Lipopolysaccharide is often referred to as endotoxin because of its unique place in microbial physiology and in the molecular pathogenesis of sepsis. Lipopolysaccharide is the major structural component of the outer membrane of Gram-negative bacteria and accounts for approximately 70% of the outer leaflet. It is essential for cell viability for virtually all Gram-negative bacterial pathogens, with the exception of one strain of *Neisseria meningitidis*.<sup>12</sup> Despite the well-known injurious host response to even minute amounts of endotoxin, lipopolysaccharide has no intrinsic toxic properties by itself.<sup>13</sup> The toxicity of lipopolysaccharide is related to the host response to this microbial mediator. Similar pathogen-associated molecular pattern mediators exist in Gram-positive bacteria and fungi that induce a potentially harmful host response during severe sepsis.

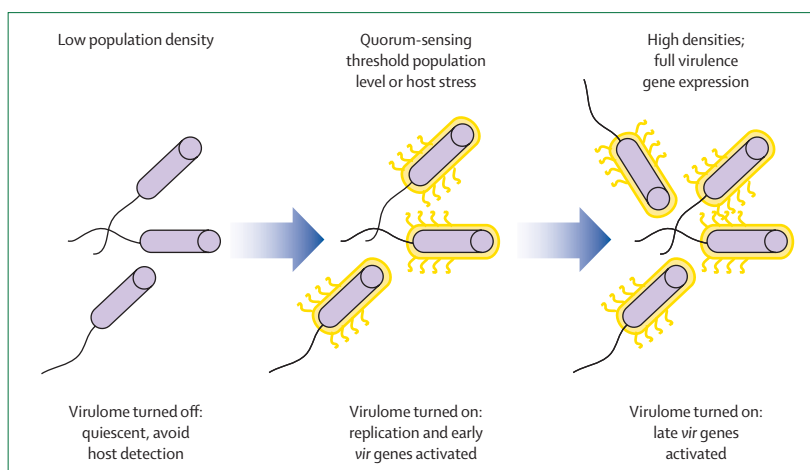
Superantigens produced by *Streptococcus* spp and *S aureus* have a prominent role in the pathogenesis of toxic shock syndromes. These unusual type I toxins are known as superantigenic because they activate CD4 T-cell populations at a level that is least five orders of magnitude greater than conventional antigens.<sup>10</sup> Superantigens are not processed for clonotypic presentation by antigen-presenting cells. They bind directly to MHC class II molecules expressed on antigen-presenting cells and cross link with a large number of T cells that bear common V $\beta$  chains and their T-cell receptor. High concentrations of lymphokines and monokines result and induce toxic

shock syndrome. Immune activation induced by superantigens potentiates the host response to other microbial mediators, including bacterial endotoxin.<sup>14</sup>

### Genomic islands, integrons, and the packaging of virulence genes

Complete genomic analyses of various microbial pathogens show that many virulence factors are packaged together in specific sequences of chromosomal DNA from which they act in concert to cause disease. Pathogenicity islands (now known simply as genomic islands) are unique sequences of DNA found in both Gram-positive and Gram-negative bacteria,<sup>15,16</sup> and probably evolved from temperate bacteriophages. They often reside adjacent to homologous regions of DNA near the genes for transfer RNA or ribosomal RNA, and are flanked by inverted or direct repeat sequences of DNA reminiscent of insertion sites for bacteriophages. Additionally, the guanine–cytosine (G–C) ratio of pathogenicity islands differs from the G–C ratio found in other regions of the bacterial chromosome. This indicates that these sequences have been horizontally transferred, and they are derived from a different genetic origin from the rest of the genome.<sup>17</sup>

Essentially, all known streptococcal and staphylococcal superantigens are associated with pathogenicity islands. Gram-negative bacteria are replete with pathogenicity islands and their presence distinguishes pathogens from avirulent strains within the same species.<sup>18</sup> Most bacterial toxins and their delivery systems (ie, type III secretion systems) are found either within lysogenic bacteriophage DNA sequences or pathogenicity islands encoded by the bacterial pathogen. Other genes found within these islands mediate inhibition of host-defence mechanisms, invasion genes, and adhesive molecules. Strong selection pressures promote the clustering of virulence genes into tightly linked sequences so they can be co-regulated and function in concert to cause disease.



**Figure 1: The central role of quorum sensing in microbial pathogenesis and virulence**

Regulation of the bacterial virulome by quorum sensing. Early virulence (*vir*) genes include adhesins, invasion genes, and expression of anticomplement and antiphagocytic measures. Late *vir* genes include exotoxins, superantigens, cytotoxins, replication activation, genetic exchange, and antibiotic resistance expression.

The adverse clinical consequences of evolutionary changes within pathogenicity islands have recently been shown by an outbreak of severe antibiotic-related colitis.<sup>19</sup> The current epidemic of severe *Clostridium difficile*-related colitis now spreading across North America and Europe is attributable to a deletion mutation within the coding sequence of a regulatory gene found in the *C difficile* pathogenicity island responsible for enterotoxin (toxin A) and cytotoxin (toxin B) expression. This deletion mutation derepresses toxin A and toxin B synthesis by this epidemic strain and increases production of these very potent toxins 16–23 times.<sup>19</sup> This epidemic is particularly severe in elderly patients and is now recognised as a cause of abdominal sepsis and death in hospital inpatients.<sup>20</sup>

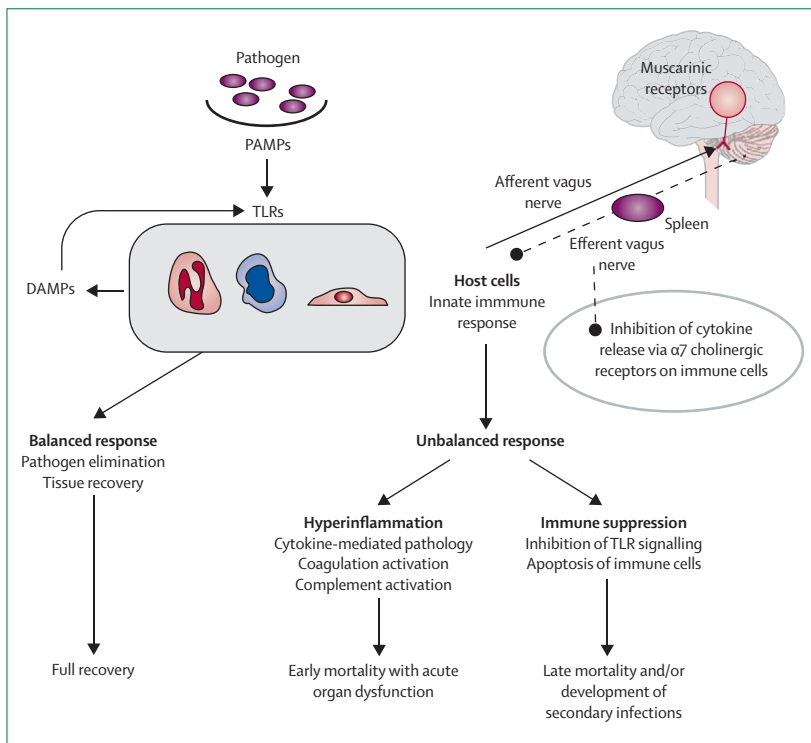
Some pathogenicity islands possess integrons, which are specialised sequences of DNA that allow the exchange of virulence genes or antibiotic-resistance genes into discrete cassettes inserted between short spacer sequences. Integrons provide a mechanism to rapidly acquire favourable genes, thereby increasing the fitness

of the organism as a human pathogen.<sup>21</sup> The recent epidemic of community-acquired methicillin-resistant *S aureus* is an excellent example of the continuing evolution of microbial pathogens. A recent clone (USA clone 300) and related isolates have adapted to the widespread use of beta-lactam antibiotics in the community by acquisition of a new genomic island (staphylococcal cassette chromosome type IV). This genetic element contains the *mecA* gene, which mediates the synthesis of low-affinity penicillin-binding proteins (ie, PBP2a) responsible for methicillin resistance. This bacterium has also acquired the genes for the expression of a Panton-Valentine leukocidin toxin, along with many other toxins and virulence factors. This clone is now capable of invasive infections in normal hosts, along with resistance to standard antimicrobial agents,<sup>22</sup> and is recognised as a cause of sepsis from necrotising soft-tissue infections and a highly destructive form of community-acquired pneumonia. Staying ahead of the pathogens responsible for sepsis will remain a major challenge for clinicians because pathogens are quite capable of rapid adaptation to antibiotic selection pressures and various other environmental changes imposed on them with new developments in modern health-care systems.<sup>23</sup>

### Bacterial cooperation and coordinated attack patterns

Quorum sensing (the ability of bacteria to assess their population density) is now recognised as a major virulence property (figure 1). Originally described in the bioluminescent, marine bacterium *Vibrio fischeri*, homologues of the quorum-sensing systems (QSSs) are now widespread among common bacterial pathogens capable of inducing severe sepsis in human beings.<sup>9,24–26</sup> Quorum sensing is crucially important in regulating population density and growth rates within biofilms. Biofilm formation is omnipresent in patients who have bacteria-colonised mucosal surfaces or medical devices (eg, vascular catheters, urinary catheters). These biofilms exist as complex and well-regulated bacterial communities, fixed to the underlying surfaces, and are relatively immune to host clearance mechanisms, at least in part by their interference with bacterial opsonisation.<sup>27</sup> Regrettably, biofilms provide a safe haven against antibiotics, because sessile bacteria within biofilms are not susceptible to the lytic effects of many classes of antimicrobial agents. Recently, the QSS has been found to have a crucial role in regulating tissue invasion by bacterial pathogens, and inhibitors of quorum sensing provide new avenues for intervention against invasive pathogens. The level of sophistication in communication between these unicellular organisms is truly remarkable. Evidence now exists that QSSs can even open up bidirectional lines of communication between bacteria and the human host.<sup>28</sup>

Many Gram-negative bacteria use a QSS similar to *V fischeri*. The QSS mediates the synthesis of an unusual acyl-homoserine lactone (AHL) moiety that functions as



**Figure 2: Host response to sepsis**

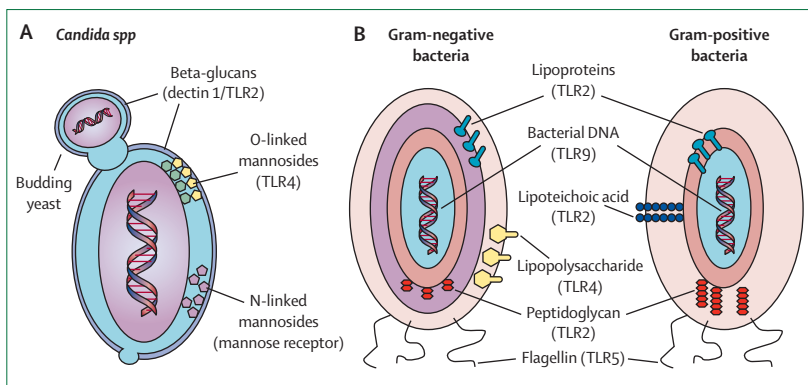
The interaction between pathogens and the host is mediated initially via an interaction between pathogen-associated molecular patterns (PAMPs) and Toll-like receptors (TLRs). This interaction can result in the release of alarmins or danger-associated molecular patterns (DAMPs), which have the ability to further amplify the inflammatory response, at least in part, via TLRs. The initial inflammation activates afferent signals that are relayed to the nucleus tractus solitarius; subsequent activation of vagus efferent activity, mediated by central muscarinic receptors in the brain, inhibits cytokine synthesis via pathways dependent on the  $\alpha 7$  subunit of acetylcholine receptors on macrophages and other cells through the cholinergic anti-inflammatory pathway (the inflammatory reflex). The resulting innate response of immune cells can result in a balanced reaction leading to pathogen elimination and tissue recovery, or an unbalanced reaction that on the one hand can lead to exaggerated inflammation and tissue injury, and on the other hand to immune suppression caused by immune-cell apoptosis and enhanced expression of negative regulators of TLR signalling.

the indicator molecule. This molecule freely diffuses across bacterial and human cell membranes. When bacterial population densities are low, limited amounts of AHSL are available and the genes under QSS control are turned off. When population densities increase beyond a threshold level, enough AHSL is generated to bind to a cytoplasmic corepressor molecule known as LuxR. This binary complex is a transcriptional activator that binds to promoter sites of gene loci under QSS regulatory control. Up to 15% of open reading frames of bacterial pathogens are under QSS control. Many phenotypic traits in several species of bacteria are under QSS control, including biofilm formation, sporulation, replication, virulence expression, genetic exchange, and antibiotic synthesis and resistance expression.<sup>29</sup>

Gram-positive pathogens also possess a functionally similar system of global regulation of genes based on cell densities.<sup>9,25</sup> Gram-positive pathogens rely on short cyclical peptides known as autoinducer indicator molecules. Cell surface receptors sense these peptides and activate a kinase that generates transcriptional activators for multiple gene loci. A third hybrid system exists and is used by Gram-negative and Gram-positive bacteria with complex, multiple-ringed, cyclical molecules as cell-density indicator molecules. This system also regulates global and coordinated transcriptional responses.

Direct evidence for an essential role of quorum sensing in microbial pathogenesis comes from site-specific quorum-sensing gene deletion experiments. *P. aeruginosa* strains with excision of the quorum-sensing gene complex lose virulence in animal models of invasive infections (eg, burns, pneumonia, bacteraemia). Full virulence is restored by inserting plasmids that carry the genes for quorum sensing back into the pathogen.<sup>25,29</sup> QSSs provide an opportunity for pathogens to minimise early losses and maximise the chances for ultimate success in causing widespread infection and sepsis. Virulence genes under QSS control are turned off when population densities are low. This limits the risk of early detection and avoids the generation of antibodies against these virulence factors in the early phases of colonisation of the host when microbial numbers are low. Once the population density expands to critical threshold levels, QSSs activate replication programmes and the full expression of virulence genes proceeds with tissue invasion.

QSS-mediated virulence gene regulation is fine tuned in some strains of *S. aureus* in which the specific sets of virulence gene transcriptional programmes are phased in and phased out in preset patterns over the course of an invasive infection. Once the QSS apparatus is activated, sequential gene activation proceeds with initial production of surface adherence molecules and tissue invasion genes. This is later followed by activation of replication systems, exotoxin synthesis, and the expression of antiphagocytic capsular components.<sup>9</sup>



**Figure 3: Innate recognition of pathogens by Toll-like (and related) receptors (TLRs)**

(A) The complexity of the interaction between innate immune receptors and fungi. Three distinct components of the cell wall of *Candida albicans* are recognised by four different host receptors: N-linked mannosyl residues are detected by the mannose receptor, O-linked mannosyl residues are sensed by TLR4, and  $\beta$ -glucans are recognised by the dectin 1–TLR2 complex.<sup>40</sup> (B) Gram-positive and Gram-negative bacteria are recognised by partly overlapping and partly distinct repertoire of TLRs. Gram-positive pathogens exclusively express lipoteichoic acid, Gram-negative pathogens exclusively express lipopolysaccharide; common pathogen-associated molecular patterns include peptidoglycan, lipoproteins, flagellin, and bacterial DNA.

QSSs provide further selective advantages for those pathogens that possess these systems. The AHSL-sensing molecules of Gram-negative bacteria inhibit the growth and survival potential of some strains of *S. aureus*,<sup>30</sup> and even the eukaryotic fungal pathogen *Candida albicans*. This would be a clear survival advantage for the bacterial strain possessing the QSS apparatus in tissue sites with complex and competing microbial communities such as mucosal surfaces. Eliminating microbial competition allows QSS-bearing bacteria to occupy favoured niches with the human host as a staging area for microbial invasion.<sup>28,30</sup>

More complex pathways of communication exist, including two-way signalling between human beings and QSS expression among bacterial populations. That such a system exists is shown by recent experiments that identify QSS-dependent alterations of multiple genetic programmes in patients.<sup>28</sup> AHSL molecules bind to intracellular signalling proteins that transcriptionally regulate the human genes that mediate the host response to bacterial invasion, such as chemokines and cytokines. This ability of some bacterial pathogens to directly regulate human immune-response genes is a clear advantage for the microorganism in this host–pathogen interaction.

Perhaps one of the most surprising findings is the capacity of human stress molecules to be recognised by the QSSs of enteric bacteria and *P. aeruginosa*.<sup>31,32</sup> A specific receptor for human interferon  $\gamma$  exists on the outer membrane (OmpF) of some pseudomonas strains that activate a series of QSS-regulated virulence genes. Excess concentrations of interferon  $\gamma$  signify a compromised and possibly vulnerable host. Activation of virulence genes and invasive phenotypes at times of host stress tips the balance between septic host and pathogen in favour of the infecting microorganism. Thus, quorum

	Species	TLR
<b>PAMPs in bacteria</b>		
Lipopolysaccharide	Gram-negative bacteria	TLR4
Lipoteichoic acid	Gram-positive bacteria	TLR2*
Peptidoglycan	Most bacteria	TLR2
Triacyl lipopeptides	Most bacteria	TLR1 or TLR2
Diacyl lipopeptides	<i>Mycoplasma</i> spp	TLR2 or TLR6
Porins	<i>Neisseria</i>	TLR2
Flagellin	Flagellated bacteria	TLR5
CpG DNA	All bacteria	TLR9
Unknown	Uropathogenic bacteria	TLR11†
<b>PAMPs in fungi</b>		
Zymosan	<i>Saccharomyces cerevisiae</i>	TLR2 or TLR6
Phospholipomannan	<i>Candida albicans</i>	TLR2
Mannan	<i>Candida albicans</i>	TLR4
O-linked mannosyl residues	<i>Candida albicans</i>	TLR4
β-glucans	<i>Candida albicans</i>	TLR2‡
<b>DAMPs§</b>		
Heat shock proteins	Host	TLR4
Fibrinogen, fibronectin	Host	TLR4
Hyaluronan	Host	TLR4
Biglycans	Host	TLR4
HMGB1	Host	TLR4, TLR2

Pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) with likely relevance for sepsis (PAMPs expressed by viruses and parasites are not shown). \*For detection of lipoteichoic acid from some pathogens, TLR6 functions as a co-receptor for TLR2. †Not functional in human beings. ‡In collaboration with dectin 1. §Recent studies describe a role for TLRs in acute injury by use of 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.<sup>41</sup> Endogenous mediators are identified as TLR4 ligands. HMGB1=high-mobility group box 1 protein.

**Table 1: Pathogen-associated and danger-associated molecular patterns and their recognition by Toll-like receptors (TLRs)**

sensing serves many functions for bacterial pathogens, and provides a system to coordinate the expression of virulence on the basis of cell densities in many common and medically important bacteria. Understanding these signalling pathways might provide new treatment options to disarm potential pathogens and improve the outcome in septic patients.<sup>33</sup>

## The host: new mediators implicated in the pathogenesis of sepsis

### Historical perspective

The assumption that sepsis is the consequence of an overwhelming inflammatory reaction of the patient to microorganisms was widely accepted for many years. This theory was based on studies in animals infused with large doses of bacteria or bacterial products. Such infusions result in a brisk systemic release of an array of inflammatory mediators, many of which have been found to be directly responsible for the death of the host, including the prototypic proinflammatory cytokines tumour necrosis factor (TNF)  $\alpha$  and interleukin 1.<sup>34-37</sup> We

now know that virtually all clinical sepsis trials with anti-inflammatory therapies failed to alter the outcome of patients with sepsis. A recurring theme in animal models of sepsis and in large clinical trials is that the incremental benefits (if any) of experimental agents accrue as the severity of the septic process increases.<sup>38</sup> Less severely ill patients with sepsis either fail to benefit or may be worsened by interventions with anti-inflammatory agents. Clearly, the hypothesis that excessive inflammation is the main underlying cause of an adverse outcome in a septic patient requires reconsideration: the host response to sepsis involves many subsequent and concurrent processes that involve both exaggerated inflammation and immune suppression (figure 2).

### Pathogen recognition systems

The innate immune system is able to detect pathogens via a limited number of pattern-recognition receptors (PRRs).<sup>39</sup> PRRs recognise conserved motifs that are expressed by pathogens but are absent in higher eukaryotes; these microbial components are known as pathogen-associated molecular patterns (PAMPs; figure 3). Additionally, PRRs may warn the host of danger in general by their ability to recognise endogenous mediators released during injurious processes, such as trauma, ischaemia, or necrosis.<sup>41</sup> Such endogenous danger signals have been termed “alarmins” or danger-associated molecular patterns (DAMPs).<sup>42</sup>

The Toll family of receptors have a central role as PRRs in the initiation of cellular innate immune responses.<sup>39,43</sup> These receptors were first discovered in the fruit fly, and 13 mammalian homologues of drosophila Toll-like receptors (TLRs 1 to 13) have been identified to date. Of these, human beings (but not mice) express TLR10, whereas mice (but not human beings) express TLR11, TLR12, and TLR13. All TLRs are single-spanning transmembrane proteins with leucine-rich repeat extracellular domains and with a cytoplasmic part largely composed of the Toll interleukin-1 receptor resistance (TIR) domain. TLRs can be expressed on the cell surface (TLRs 1, 2, 4, 5, 6, and 10) or in intracellular compartments, in particular within the endosomes (TLRs 3, 7, 8, and 9). The entire TLR family signals via four adaptor proteins: myeloid differentiation primary-response protein 88 (MyD88); TIR-domain-containing adaptor protein (TIRAP); TIR-domain-containing adaptor-protein-inducing interferon  $\beta$  (TRIF); and TRIF-related adaptor molecule (TRAM). Working in concert with several intracellular protein kinases, these TLRs recognise and respond to a myriad of highly conserved microbial molecules. Importantly, TLR signalling is tightly regulated to avoid detrimental inflammatory responses; as such, several negative regulators of TLRs have been identified including MyD88 short, interleukin-1 receptor-associated kinase (IRAK) M, ST2, single-immunoglobulin interleukin-1-receptor-related molecule (SIGIRR), and Toll-interacting protein (TOLLIP).<sup>44</sup> Given

their central role in the recognition of microbes, TLRs are likely to have a crucial role in sepsis: TLRs are on the one hand essential for the early detection of pathogens, but on the other hand cause excessive inflammation after uncontrolled stimulation. TLRs may further contribute to the pathogenesis of sepsis by amplifying inflammatory responses by interaction with DAMPs released after tissue injury; in this respect TLR4 seems to be of particular importance.<sup>41</sup> Table 1 summarises PAMPs and DAMPs with (likely) relevance for the pathogenesis of sepsis and their interaction with TLRs.

TLRs detect pathogens at either the cell surface or in lysosomes or endosomes. Pathogens that invade the cytosol are recognised by various cytoplasmic PRRs. Nucleotide-binding oligodimerisation domain (NOD) proteins NOD1 and NOD2 contribute to the detection of common fragments of peptidoglycan (ie, diamino-pimelate for NOD1, and muramyl dipeptide for NOD2) in the cytosol.<sup>39</sup> Additionally, bacterial infection leads to activation of caspase 1 in a protein complex termed the NOD-like receptor (NLR) family pyrin-domain-containing 3 (NLRP3) inflammasome.<sup>45,46</sup> NLRP3 (also known as cryopyrin) regulates the activity of caspase 1, an enzyme responsible for the secretion of three interleukin-1 family members implicated in host defence against infection: interleukin 1 $\beta$ , interleukin 18, and interleukin 33. Caspase 1 and its proinflammatory cytokine products are likely to contribute to the pathogenesis of sepsis in overwhelming inflammation, such as induced by bolus injection of high-dose lipopolysaccharide,<sup>47–49</sup> although it has a positive impact on host defence against several infections.<sup>49,50</sup> The potential deleterious or advantageous role of caspase 1 resembles the bimodal roles of TLR2 and TLR4 as a part of the early warning system against microbial invasion, even though they also contribute to the initiation of sepsis. A vigorous innate immune response is now recognised as a double-edged sword, with a crucial role in defending the host through activation of antimicrobial defences, and yet, if left unchecked, the same system contributes to systemic inflammation, intravascular coagulation, tissue injury, and death caused by severe sepsis.

### Coagulation and anticoagulation

Patients with sepsis almost invariably show evidence of activation of the coagulation system. Several clinical studies have suggested that sepsis-related disseminated intravascular coagulation is associated with not only high mortality but also organ dysfunction, and that attenuation of coagulation may ameliorate organ failure in this condition.<sup>51–53</sup>

Tissue factor is regarded as the primary initiator of coagulation in sepsis.<sup>54,55</sup> Tissue factor is constitutively expressed in the extravascular compartment to initiate clotting if blood leaves the confines of the endothelial surface. During severe sepsis, activated monocytes and

endothelial cells, along with circulating microvesicles, become sources of tissue factor. Human beings intravenously injected with lipopolysaccharide rapidly increase tissue factor mRNA concentrations in circulating blood cells and release tissue-factor-containing microparticles.<sup>56,57</sup> Inhibitors of the factor VIIa–tissue factor pathway in experimental studies in human beings and primates abrogate the activation of the common pathway of coagulation.<sup>58–61</sup>

Blood clotting is controlled by three major anticoagulant proteins: tissue-factor-pathway inhibitor (TFPI), antithrombin, and activated protein C (APC).<sup>54,55</sup> TFPI is an endothelial-cell-derived protease inhibitor that blocks the activity of factor Xa when bound to factor-VIIa–tissue-factor complex. Antithrombin inhibits factor Xa, thrombin, and factor IXa, as well as factor-VIIa–tissue-factor complex. The protein-C–protein-S system attenuates coagulation by the capacity of APC to proteolytically inactivate factors Va and VIIIa. Haemostasis is further controlled by the fibrinolytic system, in which plasminogen activator inhibitor type 1 (PAI-1) functions as a major inhibitor. Notably, during severe sepsis, the activities of TFPI, antithrombin, the protein-C–APC system, and fibrinolysis are impaired, resulting in a net procoagulant state.<sup>62</sup> In septic primates, the administration of either TFPI, antithrombin, or APC attenuated consumptive coagulopathy,<sup>60,63,64</sup> and large clinical trials in patients with sepsis have been completed.<sup>65–68</sup> Only APC was found to reduce 28-day mortality significantly in patients with severe sepsis;<sup>65</sup> importantly, APC was not effective in those patients with severe sepsis who had a low risk of death.<sup>68</sup> Furthermore, in a recent placebo-controlled trial in 477 children with sepsis-induced cardiovascular and respiratory failure, recombinant human APC did not influence the composite time to complete organ failure resolution or 28-day mortality.<sup>69</sup> Of note, the European licensing authorities have recently asked Eli Lilly to do another placebo-controlled trial with APC in adult patients with severe sepsis.

PAI-1 has been implicated in the pathogenesis of sepsis because elevated circulating PAI-1 concentrations are highly predictive for an unfavourable outcome in sepsis patients.<sup>70</sup> Additionally, a sequence variation in the gene encoding PAI-1 influences the development of septic shock in patients with meningococcal infection.<sup>71</sup> Recently, studies using PAI-1-deficient mice and mice with transiently enhanced expression of PAI-1 have pointed to a protective rather than a harmful role of this mediator in severe Gram-negative pneumonia and sepsis.<sup>72</sup> Further studies are warranted to confirm such a role for PAI-1 in other models of sepsis.

### Immune suppression and apoptosis

Patients who have survived the initial phase of sepsis show features consistent with immunosuppression.<sup>73–75</sup> The timing of the first occurrence of immunosuppression

in sepsis is a matter of debate: some investigators favour the subsequent initiation of an hyperinflammatory and anti-inflammatory response, whereas others have suggested that immunosuppression is a primary rather than a compensatory response of sepsis.<sup>73–76</sup> Many studies have reported the reduced capacity of circulating leucocytes obtained from sepsis patients to release proinflammatory cytokines. Although the mechanisms that underlie this phenomenon have not been fully elucidated, anti-inflammatory cytokines, particularly interleukin 10 and transforming growth factor  $\beta$ , are probably involved. Additionally, negative regulators of TLR signalling may play a part.

Deregulated apoptotic immune-cell death has been implicated to play a major part in immune dysfunction and mortality in sepsis.<sup>74,75,77</sup> Apoptosis is a physiological

process by which cells are eliminated in a controlled manner (programmed suicide) to limit damage of surrounding tissue. Apoptotic cells produce anti-inflammatory cytokines and elicit anergy, which impairs the response to pathogens; necrotic cells cause immune stimulation and enhance defence against microbial pathogens.<sup>78,79</sup> Most cells that undergo enhanced apoptosis in sepsis are of lymphoid origin. Necropsies done on patients within 30–90 min after death caused by sepsis have disclosed a profound apoptosis-induced loss of B cells, CD4 T cells, and follicular dendritic cells, along with gastrointestinal epithelial cells.<sup>80,81</sup> The pathogenetic significance of these findings has been shown in animal models of sepsis, in which prevention of apoptosis of lymphocytes or the intestinal epithelium improved survival.<sup>82–84</sup> In a novel approach to inhibit apoptosis,

	Target	Sponsor or institution	Phase	Comments
Lipid emulsion (GR-270773)	TLR4	GlaxoSmithKline	II	Completed, showed no differences between treatment groups with respect to 28-day mortality
E5564 (Eritoran)	TLR4	Eisai	III	..
TAK-242	TLR4	Takeda	II	Study was stopped after a planned stopping point. No results are available. Takeda is planning a new phase II or III study
Unfractionated heparin	Coagulation	Universidad de Antioquia, Colombia	III	..
Recombinant antithrombin	Coagulation	LeoPharma	II	..
Recombinant activated protein C	Coagulation	Eli Lilly	III/IV	Recombinant activated protein C (drotrecogin alfa activated) is an approved drug for sepsis, but recently the European regulatory agency have requested another placebo-controlled trial in high-risk patients with severe sepsis
Recombinant tissue-factor pathway inhibitor	Coagulation	Novartis	III	Tissue factor pathway inhibitor is currently in clinical trial for severe community-acquired pneumonia; most of these patients would meet sepsis criteria
Intensive insulin therapy	Hyperglycaemia	German Competence Network Sepsis, Germany	III	This trial (VISEP trial) compared both two strategies of volume substitution (colloid vs crystalloid) and intensive versus conventional insulin treatment. Intensive insulin therapy was reported to reduce mortality in patients admitted to a primarily surgical ICU <sup>118</sup> and in patients admitted to a medical ICU who remained there for at least 3 days. <sup>119</sup> Whereas these studies did not focus on sepsis patients, this recent German study (unpublished) was stopped because of no apparent benefit and a high incidence of hypoglycaemia
Corticosteroids	Adrenal suppression	Hadassah Medical Organization, Israel	III	Steroid therapy for severe sepsis has been the subject of debate for decades. The recent CORTICUS trial (not yet published) with stress-dose glucocorticoids will not end the debate
Hydrocortisone vs hydrocortisone+fludrocortisone	Adrenal suppression	Charleston Area Medical Centre Health System, WV, USA	IV	..
Intensive insulin therapy and hydrocortisone vs hydrocortisone+fludrocortisone	Hyperglycaemia and adrenal suppression	University of Versailles, France	III	..
Granulocyte-macrophage colony-stimulating factor	Immunoparalysis	Charité University, Berlin, Germany	II	..
Albumin	Shock	Laboratoire Français de Fractionnement et de Biotechnologies, Paris, France	IV	..
Rosuvastatin	Unknown	Universidad Autonoma de San Luis Potosi, Mexico	II	Human data hint at reduced mortality rates in bacteraemic patients, and a reduced risk of sepsis in patients with bacterial infections concurrently taking statins. <sup>120</sup> These lines of evidence resulted in phase II trials to assess the statins rosuvastatin, atorvastatin, and simvastatin in patients with sepsis
Atorvastatin	Unknown	Hospital de Clinical de Porto Alegre, Brazil	II	As above
Simvastatin	Unknown	Medical University of Vienna, Austria	IV	As above. This trial is not yet open for recruitment of patients

Data were obtained from <http://www.clinicaltrials.gov> (as of June 4, 2007). ICU=intensive care unit; TLR=Toll-like receptor.

Table 2: Novel anti-sepsis strategies currently investigated in clinical trials

hydrodynamic administration of small-interfering RNA against the death receptor Fas or caspase 8 decreased apoptosis in tissues and improved the survival of mice after caecal ligation and puncture.<sup>85</sup>

Apoptosis inhibitors have not been tested in patients with sepsis. Potential problems include the selectivity of such inhibitors and the risk of uncontrolled cell growth. Moreover, apoptosis is an important mechanism for eliminating activated neutrophils from inflamed tissues; because continuing accumulation of neutrophils in tissues may be linked to development of organ injury, caution is warranted before the use of apoptosis inhibitors in clinical sepsis. Other strategies to restore immune function include the administration of immunostimulating cytokines. In a small uncontrolled study in nine patients, daily subcutaneous injection of interferon  $\gamma$  restored the TNF $\alpha$  production capacity of monocytes; although the efficacy of interferon  $\gamma$  could not be determined, eight patients recovered from sepsis shortly after treatment.<sup>86</sup>

#### High-mobility group box 1 protein

High-mobility group box 1 protein (HMGB1) is a nuclear protein present in almost all eukaryotic cells, where it functions to stabilise nucleosome formation. HMGB1 is released from necrotic cells, as well as from macrophages, dendritic cells, and natural killer cells, on activation by infectious agents.<sup>87</sup> HMGB1 is a late-acting pro-inflammatory cytokine in the pathogenesis of sepsis, as shown by serial measurements in experimental settings in which HMGB1 is detected only after more than 8 h.<sup>88,89</sup> An anti-HMGB1 antibody protected against lipopolysaccharide-induced death in mice even after the peak concentrations of TNF $\alpha$  and interleukin 1 had been reached.<sup>88</sup> Anti-HMGB1 treatment increased survival in mice with caecal ligation and puncture when given 24 h after the surgical procedure.<sup>89</sup> Increased HMGB1 concentrations are readily detected in patients with sepsis.<sup>88,90</sup> Of note, HMGB1 acts downstream of cell apoptosis during severe sepsis.<sup>91</sup> Indeed, during sepsis induced by caecal ligation and puncture, macrophages released HMGB1 on exposure to apoptotic cells, and a monoclonal anti-HMGB1 antibody conferred protection without influencing the accumulation of apoptotic cells in the spleen.<sup>91</sup> Considering that the therapeutic window for anti-HMGB1 therapies should be much wider than for TNF-neutralising strategies, inhibitors of HMGB1 may be valuable adjunct for established severe sepsis.

Whether highly purified HMGB1 can directly activate cells is not certain.<sup>92</sup> HMGB1 may function as a carrier protein that brings other mediators to target cells. Several receptors have been identified as possible receptors for the cellular effects of HMGB1, including TLR2 and TLR4, and the receptor for advanced glycation end-products (RAGE).<sup>87</sup> RAGE is a ubiquitous receptor that recognises diverse endogenous ligands, such as advanced glycation end-products, S100/calgranulins, amyloid A, leucocyte

adhesion receptors, *E coli* curli operons, and HMGB1. RAGE ligation can activate nuclear factor  $\kappa$ B and mitogen-activated protein kinase pathways.<sup>93</sup> The potential role of RAGE signalling in sepsis pathophysiology has been reported in mice exposed to caecal ligation and puncture: RAGE-deficient mice and wild-type mice treated with soluble RAGE were partly protected against death from severe sepsis.<sup>94</sup> Further research is warranted to address the therapeutic potential of RAGE (ligand) inhibitors in sepsis.

#### Cholinergic anti-inflammatory pathway

The cholinergic nervous system, and in particular the vagus nerve, plays an important part in limiting inflammatory responses.<sup>95,96</sup> In the cholinergic anti-inflammatory pathway, enhanced efferent activity of parasympathetic nerve endings results in the release of acetylcholine, which suppresses proinflammatory cytokine production by a specific action on  $\alpha$ 7 cholinergic receptors on macrophages.<sup>97</sup> Disruption of this neural-based system by vagotomy renders animals more vulnerable to the toxic effects of lipopolysaccharide: in rats, surgical dissection of the vagus nerve led to exaggerated release of TNF $\alpha$  and accelerated hypotensive shock after intravenous injection of lipopolysaccharide;<sup>98</sup> vagotomy also enhanced the local and systemic inflammation accompanying bacterial peritonitis.<sup>99</sup> Conversely, electrical stimulation of the efferent vagus nerve prevented the development of shock and attenuated the release of TNF $\alpha$  and the activation of the coagulation system in endotoxaemic rats,<sup>98,100</sup> whereas stimulation of  $\alpha$ 7 cholinergic receptors by specific agonists, such as nicotine, attenuated systemic inflammation and improved the outcome of mice with polymicrobial abdominal sepsis.<sup>101</sup> Recent evidence indicates that, within the brain, central muscarinic receptors play a part in activating the cholinergic anti-inflammatory pathway,<sup>102</sup> and that the spleen is an essential peripheral part of the cholinergic anti-inflammatory reflex.<sup>103</sup> Together, these preclinical data suggest that stimulation of the vagus nerve or pharmacological  $\alpha$ 7 cholinergic receptor agonists, or both, may be useful strategies in the treatment of the severe inflammation that accompanies sepsis.

#### Search strategy and selection criteria

Data for this Review were identified by searches of PubMed and references from relevant articles. Articles were also identified through searches of the extensive files of the authors. Search terms used were "bacterial virulence", "quorum sensing", "pathogenicity islands", "pathogenesis of sepsis", "bacterial toxins", "endotoxin", "superantigens", "Toll-like receptors", "sepsis AND coagulation", "sepsis AND apoptosis", and "sepsis AND complement". Only English language articles were reviewed. No date restrictions were set in these searches.



### Macrophage migration inhibitory factor

Macrophage migratory inhibitory factor (MIF) is a cytokine produced by many different cell types. Glucocorticoids act as inducers of MIF production by macrophages,<sup>104</sup> and serum MIF concentrations are increased in patients with sepsis.<sup>105</sup> Evidence in support of MIF as a contributor to the pathogenesis of sepsis includes the following: (1) inhibition or elimination of MIF protected mice from death from lipopolysaccharide or abdominal sepsis;<sup>105,106</sup> (2) administration of MIF increased risk of death after lipopolysaccharide challenge;<sup>105,106</sup> and (3) genetic deletion of MIF in mice resulted in a decrease in the production of proinflammatory mediators, including TNF $\alpha$  and interleukin 1 $\beta$ .<sup>106</sup> MIF might participate in the resolution of inflammation by its unique ability to regulate activation-induced apoptosis.<sup>107</sup> In the presence of high concentrations of MIF, the timely removal of activated monocytes/macrophages by apoptosis is suppressed, allowing enhanced monocyte/macrophage survival, increased cytokine production, and a sustained proinflammatory response. MIF enhances macrophage expression of TLR4, thereby further influencing innate immunity.<sup>108</sup> These data suggest that MIF could be an interesting target for therapeutic intervention in patients with sepsis. Of note, a recent study suggested that highly purified recombinant MIF does not exert conventional cytokine-like activity, but rather acts to modulate and amplify responses to lipopolysaccharide.<sup>109</sup>

### C5a and C5a receptor

The complement system is composed of more than 30 plasma proteins and receptors, and acts as an enzymatic cascade through various protein–protein interactions. Three pathways of complement activation have been recognised: classic, alternative, and lectin-binding pathways.<sup>110</sup> Clinical and experimental sepsis is associated with increased plasma concentrations of complement constituents C3a, C4a, and C5a. The importance of C5a for the outcome of sepsis has been underscored by several experimental investigations. Infusion of anti-C5a antibodies improved haemodynamic variables in pigs infused with lipopolysaccharide or live *E coli*,<sup>111</sup> reduced mortality in primates with *E coli* sepsis,<sup>112</sup> and improved survival in rats subjected to caecal ligation and puncture.<sup>113</sup> Additionally, the receptor for C5a is upregulated in many organs from septic animals, and anti-C5a treatment attenuated the coagulopathy of sepsis and improved organ function.<sup>114,115</sup> C5a may further harm the septic host by inhibiting neutrophil apoptosis and concurrently enhancing apoptosis of thymocytes.<sup>116,117</sup> Interventions that block C5a signalling represent promising targets for sepsis treatment. The principal therapeutic goal of complement inhibition in patients with severe infection would be to retain complement's role in host defences, while preventing the pathological activities of complement activation products.

### Conclusions

Sepsis remains a major challenge for clinicians. Microbial pathogens have proven to be more ingenious in avoiding and altering host defences than we originally anticipated. The capacity to subvert host defences, communicate with each other, and cooperate during the invasive phase of infection reveals a level of sophistication in microbial pathogenesis that is only beginning to be fully appreciated. Recent insights into the early interactions between pathogens and the host may pave the way for novel therapeutic interventions. Several interventions based on these new insights are currently being assessed in clinical trials in patients with sepsis, including inhibitors of TLR4 signalling and the immune stimulant granulocyte–macrophage colony stimulating factor (table 2). We anticipate that more novel anti-sepsis strategies will be clinically assessed in the near future.

### Conflicts of interest

TvdP is a member of the Sepsis Steering Committee of the phase III sepsis trial with E5564 (TLR4 antagonist; Eisai) and has received research support for preclinical research on activated protein C from Eli Lilly. SMO has received research support from Genetics Institute-Wyeth, is the principal investigator for the phase III sepsis trial with E5564 (TLR4 antagonist, Eisai), and a member of the Ocean State Clinical Coordinating Centre, which receives grant money to oversee the Novartis Tifacogin CAP study. Both authors are members of the Steering Committee of the International Sepsis Forum (ISF; <http://www.sepsisforum.org>), a non-profit organisation of academic physicians and industry sponsors whose principal goal is to facilitate a greater awareness of sepsis as an important clinical problem and to promote the research and development of new agents for the treatment of sepsis. The ISF receives unrestricted educational grants to achieve these goals from BRAHMS, BioMerieux, Biosite Inc, Exponential Biotherapies Inc, Eisai Inc, Eli Lilly, GlaxoSmithKline, Novo Nordisk, Roche Diagnostic GmbH, Spectral Diagnostics Inc, Takeda Pharmaceuticals North America, and Toray Medical Co.

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