

The immunopathogenesis of sepsis

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Sepsis is a condition that results from a harmful or damaging host response to infection. Many of the components of the innate immune response that are normally concerned with host defences against infection can, under some circumstances, cause cell and tissue damage and hence multiple organ failure, the clinical hallmark of sepsis. Because of the high mortality of sepsis in the face of standard treatment, many efforts have been made to improve understanding of the dysregulation of the host response in sepsis. As a result, much has been learnt of the basic principles governing bacterial–host interactions, and new opportunities for therapeutic intervention have been revealed.

Sepsis describes a complex clinical syndrome that results from a harmful or damaging host response to infection. As a result of a concerted effort to understand the underlying pathogenetic mechanisms, there have been significant advances that have illuminated not just the process of sepsis, but also fundamental principles governing bacterial–host interactions. Unfortunately, attempts to translate these observations into improved clinical outcomes proved unsuccessful and led to considerable frustration. But in the past year, four major clinical trials that are based on somewhat different strategies have shown that it is possible to significantly reduce the mortality from sepsis and septic shock, and it is therefore timely to review these developments, both in basic science and its clinical applications.

Sepsis develops when the initial, appropriate host response to an infection becomes amplified, and then dysregulated. Clinically, the onset is often insidious: features may include fever, mental confusion, transient hypotension, diminished urine output or unexplained thrombocytopenia. If untreated, the patient may develop respiratory or renal failure, abnormalities of coagulation, and profound and unresponsive hypotension. A recent epidemiological study from North America found that the incidence was approximately 3.0 cases per 1,000 population, which translates into an annual burden of approximately 750,000 cases. The overall mortality is approximately 30%, rising to 40% in the elderly and is 50% or greater in patients with the more severe syndrome, septic shock¹. It is worth emphasizing that these figures represent mortality rates in patients admitted to hospital intensive care units and given antibiotics and the best available supportive care. The commonest sites of infection are the lungs, abdominal cavity, the urinary tract and primary infections of the blood stream. A microbiological diagnosis is made in about half the cases; Gram-negative bacteria account for about 60% of cases, Gram-positive for the remainder^{1,2}.

Microbial components that initiate injury

Determining the structural components of bacteria that are responsible for initiating the septic process has been important not only in understanding the underlying mechanisms, but also in identifying potential therapeutic targets. These bacterial motifs, which are recognized by the innate immune system, have been called pathogen-associated molecular patterns (PAMPs)³, although it might be more accurate to call them microorganism-associated molecular patterns as it is by no means clear how the host distinguishes between signals from pathogens rather than commensals.

In Gram-negative bacteria, lipopolysaccharide (LPS; known also as endotoxin) has a dominant role. The outer membrane of Gram-negative bacteria is constructed of a lipid bilayer, separated from the inner cytoplasmic membrane by peptidoglycan. The LPS molecule is embedded in the outer membrane and the lipid A portion of the molecule serves to anchor LPS in the bacterial cell wall.

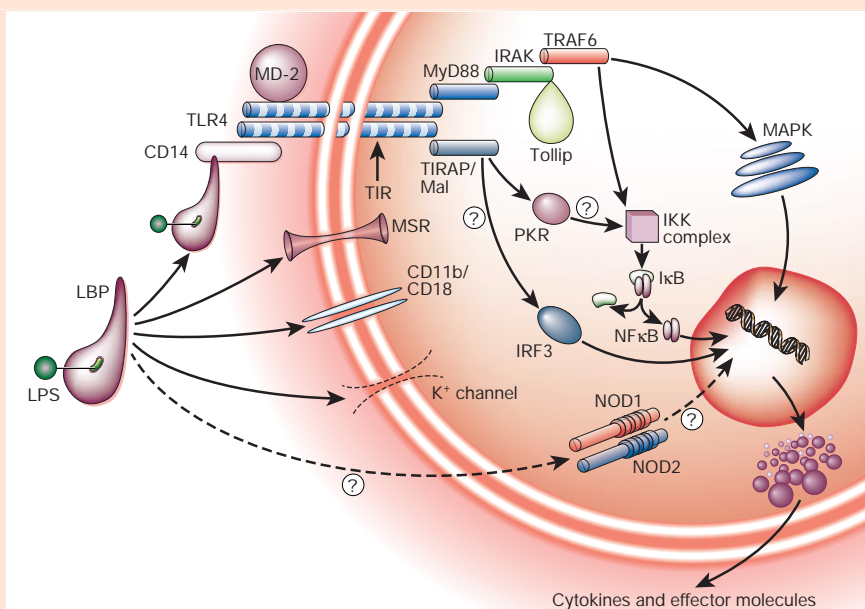
Biophysical studies on the three-dimensional conformation adopted by different lipid A partial structures have revealed that, under physiological conditions, the most active forms assume the shape of a truncated cone, whereas inactive molecules prefer a lamellar structure and become progressively more cylindrical⁴. These conformational changes seem to correlate with the ability to activate host cell membranes.

There is no endotoxin in Gram-positive bacteria, but their cell walls do contain peptidoglycan and lipoteichoic acid, and several investigators have identified structural components that account for their biological activity^{5,6}. Both peptidoglycan and lipoteichoic acid can bind to cell-surface receptors and are pro-inflammatory⁷, although they are much less active, on a weight-for-weight basis, than LPS. Their role in the pathogenesis of clinical sepsis remains uncertain because there are no convincing clinical data to show that they are present in the circulation at concentrations comparable to those used in the experimental setting.

However, an important feature of Gram-positive cells is the production of potent exotoxins, some of which are implicated in septic shock. The best known examples are the toxic shock syndromes caused by toxic shock syndrome toxin-1 (TSST-1)-producing strains of *Staphylococcus aureus* and the pyrogenic exotoxins from *Streptococcus pyogenes*. Toxic shock syndromes are among the most acute and most severe forms of septic shock. They frequently occur without warning in otherwise healthy individuals and the mortality can be as high as 50%. These Gram-positive exotoxins are of great interest because they exhibit the properties of superantigens, that is, they are able to bind promiscuously to major histocompatibility complex class II and a restricted repertoire of T-lymphocyte receptor (TCR) V β domains. In so doing they cause massive T-cell activation and release of pro-inflammatory lymphokines⁸, suggesting a plausible role for these toxins as a cause of the profound shock that is seen in patients with toxic shock.

Detailed structural analyses have been done for many bacterial superantigens, and the crystal structures of several staphylococcal and streptococcal toxins have been elucidated⁹. Interestingly, sequence variability in the amino-terminal domain dictates varying affinities for specific human leukocyte antigen (HLA) class II alleles; for instance, the strep-

Figure 1 Cell-surface recognition of lipopolysaccharide (LPS). The principal mechanism by which LPS is sensed is via an LPS-binding protein (LBP)–LPS complex and then signalling through the Toll-like receptor 4 (TLR4)–MD-2 complex. However, other cell surface molecules also sense LPS; these include the macrophage scavenger receptor (MSR), CD11b/CD18 and ion channels. Intracellular signalling depends on binding of the intracellular TLR domain, TIR (Toll/IL-1 receptor homology domain), to IRAK (IL-1 receptor-associated kinase), a process that is facilitated by two adapter proteins, MyD88 (myeloid differentiation protein 88) and TIRAP (TIR domain-containing adapter protein; also called MyD88-adapter-like protein or Mal), and inhibited by a third protein Tollip (Toll-interacting protein). Note that there is also a MyD88-independent pathway by which TIRAP/Mal signals through an RNA-dependent protein kinase (PKR) and interferon regulatory factor (IRF)-3. Recently it has been proposed that cells may also be able to respond to LPS by intracellular receptors called NOD proteins (for nucleotide-binding oligomerization domain). NOD1 (also called caspase-recruitment domain 4) was identified originally on the basis of structural homology to the apoptosis



regulator, Apaf-1. The NOD proteins have some similarities to the resistance (R) genes in plants that are involved in pathogen recognition: in common with TLRs and R genes, NODs have leucine-rich repeats. Expression of NOD1 and NOD2 confer responsiveness to Gram-negative LPS but not to lipoteichoic acid, which is found in Gram-positive bacteria. The mechanism by which NOD may recognize LPS in the cytosol is unknown.

tococcal superantigen SPEA (for streptococcal pyrogenic exotoxin A) shows significantly greater affinity for HLA-DQ than HLA-DR. These differences may in part explain the remarkable selectivity of the toxic shock syndromes: although staphylococcal and streptococcal strains bearing superantigen genes are widespread and indeed frequently cause infections, toxic shock syndromes are relatively uncommon.

Although experimental and epidemiological studies provide some support for the view that these superantigenic toxins are the cause of the toxic shock syndromes¹⁰, it is by no means clear that it is their superantigenicity *per se* that is responsible. For instance, despite many data that implicate the streptococcal toxin SPEA¹¹, this is in fact a relatively weak superantigen compared to the more recently described toxin streptococcal mitogenic exotoxin Z (SMEZ)¹². Yet in experimental models in which HLA-DQ transgenic mice are challenged with strains of *S. pyogenes* in which *smez* is disrupted, there is no effect on survival despite a profound reduction in pro-inflammatory activity¹³. These findings are important because there is considerable interest in devising therapeutic strategies that are targeted at Gram-positive infections and the toxic shock syndromes, and it is not clear whether these strategies should be aimed at the superantigenicity, or at other pro-inflammatory properties of the toxins.

There are also data that suggest that superantigenic toxins from Gram-positive bacteria induce hypersensitivity to LPS. The staphylococcal toxin TSST-1 enhances the susceptibility of rabbits to a lethal injection of LPS by a factor of approximately 50,000, and co-injection of LPS and TSST-1 induces tumour-necrosis factor- α (TNF- α) levels significantly higher than injection of similar doses of either toxin alone. Mice with severe combined immunodeficiency, lacking B and T lymphocytes, are resistant to this effect, but regain sensitivity when reconstituted with T-cells, and the mechanism seems to be dependent on enhanced production of interferon- γ (IFN- γ) from toxin-activated T cells¹⁴. This interaction between superantigens and LPS might in part explain the devastating nature of the toxic shock syndromes. It could also have therapeutic implications, as it might be advantageous to target LPS even if the infection is apparently caused exclusively by Gram-positive bacteria.

Several other bacterial components have been shown to have pro-inflammatory activity and to be able to induce shock in experimental systems. These include cell-wall structures such as flagellin¹⁵ and curli¹⁶, and unmethylated CpG sequences in naked bacterial DNA¹⁷. Receptors for some of these elements have been identified among the family of Toll-like proteins that are now known to be crucial in the cellular recognition of microbial structures¹⁸.

Host recognition of microbial components

The CD14–LBP complex

The inability to identify an ‘LPS receptor’ was for many years a barrier to understanding how Gram-negative bacteria initiated the septic response, but in a series of elegant studies it was shown that activation of host cells was dependent on the presence of LPS-binding protein (LBP) and the opsonic receptor CD14 (ref. 19). Although CD14 was originally identified as the essential co-receptor that mediated LPS activation of monocytes, subsequent work has shown that it also participates in the activation by Gram-positive cell-wall components such as peptidoglycan²⁰, mediates macrophage apoptosis²¹, and is important in shuttling LPS between serum proteins that have the capacity to bind LPS, such as LBP and serum lipoproteins²². Membrane bound CD14 (mCD14) is a glycosylphosphatidylinositol-linked molecule anchored in the cell surface, but it is also found in the circulation as soluble CD14 (sCD14). Many cells that are constitutively CD14 negative, such as dendritic cells, fibroblasts, smooth muscle cells and vascular endothelium, are still able to respond to LPS by interacting with sCD14. sCD14 is found in the serum of healthy individuals but levels rise in sepsis²³, and antibody to CD14 protects primates from lethal endotoxin shock²⁴.

Toll-like receptors

Although the discovery of CD14 represented a significant step forward in understanding host responses to LPS, the fact that mCD14 had no intracellular tail meant that it remained unclear how ligation of the LPS–LBP complex led to cellular activation. This uncertainty was resolved by the discovery of the family of Toll-like receptors

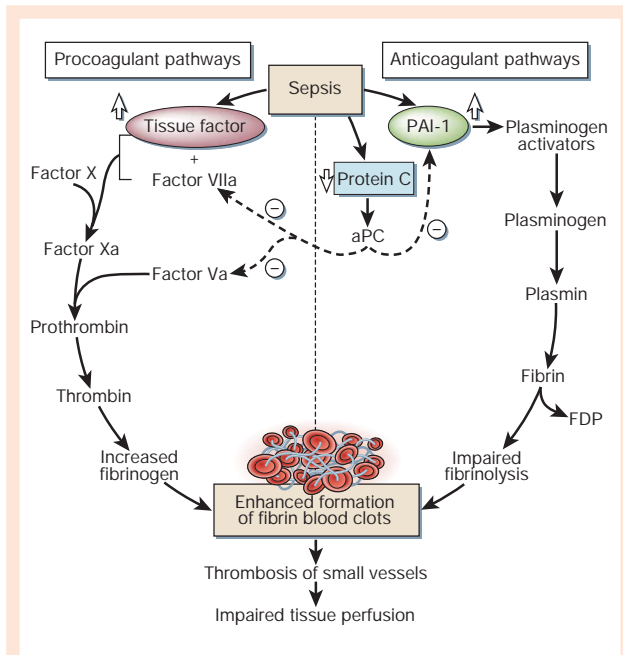


Figure 2 Sepsis disturbs the normal homeostatic balance between procoagulant and anticoagulant mechanisms. Tissue factor expression is enhanced leading to increased production of prothrombin that is converted to thrombin, and that in turn generates fibrin from fibrinogen. Simultaneously, levels of the plasminogen-activator inhibitor-1 (PAI-1) are increased, resulting in impaired production of plasmin and thus failure of normal fibrinolytic mechanisms by which fibrin is converted to degradation products (FDP). Sepsis also causes a fall in the levels of the natural anticoagulant protein C (and also antithrombin and the tissue factor pathway inhibitor, TFPI, not shown). The activated form of protein C, aPC, dissociates from the endothelial protein C receptor to inactivate factors Va and VIIa and inhibit PAI-1 activity; hence reduced levels of protein C result in further procoagulant effect. The net result is enhanced formation of fibrin clots in the microvasculature, leading to impaired tissue oxygenation and cell damage.

(TLRs)^{25,26}. Over a remarkably short period of time, studies of innate immunity in *Drosophila* revealed the existence of a proteolytic cascade that yielded ligands for cellular receptors that could distinguish bacterial from fungal infection. It was shown subsequently that there were striking similarities between this system and the interleukin (IL)-1 signalling system in mammals. This in turn led to the identification of human TLRs²⁷ and the discovery that a TLR was the long-sought co-receptor for LPS²⁸.

A family of (currently) ten TLRs has been identified with a wide range of ligand specificity including bacterial, fungal and yeast proteins^{25,29}. Thus, TLR4 is the LPS receptor, TLR2 is predominantly responsible for recognizing Gram-positive cell-wall structures³⁰, TLR5 is the receptor for flagellin³¹ and TLR9 recognizes CpG elements in bacterial DNA¹⁸. An additional cell-surface molecule, MD-2, has been identified that is required for activation of TLR4 (ref. 32). MD-2 knockout mice do not respond to LPS and survive endotoxic shock. The role of MD-2 seems to be that of positioning TLR4 correctly on the cell surface, as in MD-2^{-/-} embryonic fibroblasts TLR4 remained within the Golgi and failed to appear on the cell surface³³.

The notion of a 'monogamous' association between one particular TLR and its microbial ligand, as in the case of LPS and TLR4, is in reality an oversimplification. For instance, TLR2 can be activated by cell-wall components of both yeast and mycobacteria. Further complexity is introduced into the system by the fact that TLRs seem to be able to combine to form a repertoire capable of distinguishing closely related ligands³⁴, and there is at least preliminary evidence that polymorphisms in Toll-family proteins might provide part of the

explanation for the enormous variability in individual responses to what seem to be similar infective challenges^{35,36}.

Signalling pathways activated by TLRs have been dissected in great detail, and show a remarkable degree of homology with the Toll activation pathway in *Drosophila*³⁷. TLRs have an intracellular domain that is homologous with the IL-1 receptor and the IL-18 receptor. Adapter proteins facilitate binding to IL-1 receptor-associated kinase, which in turn induces TNF receptor-associated factor-6, leading to nuclear translocation of nuclear factor- κ B (NF- κ B) and ultimately to activation of cytokine gene promoters (Fig. 1). Although this model is based on LPS signalling of TLR4, a similar — although not identical — process is involved in the activation of TLR2 by Gram-positive bacteria.

Other host signal molecules that respond to bacteria

A further layer of complexity has been provided by the discovery that there are several additional pathways by which cells recognize microbial components. Peptidoglycan-recognition proteins (PGRPs) were identified in moths and subsequently a family of PGRP genes was found in *Drosophila*³⁸ and in humans³⁹. Different PGRPs can distinguish between Gram-positive⁴⁰ and Gram-negative bacteria^{41,42}. In *Drosophila*, they seem to act by regulating activation of Relish, a member of the NF- κ B family⁴³, although the precise mechanism by which they are sensed at the cell surface remains unknown.

The triggering receptor expressed on myeloid cells (TREM-1) and the myeloid DAP12-associated lectin (MDL-1) are two recently identified receptors involved in monocyte activation and inflammatory response. TREM-1 is upregulated in the presence of various microorganisms⁴⁴, although the ligand for TREM-1 is unknown. When mononuclear cells are exposed to a combination of LPS and an antibody to TREM-1, there is a synergistic effect and enhanced production of pro-inflammatory cytokines. But if a fusion protein of TREM-1 and the Fc portion of IgG is used to compete with cell-bound receptor, LPS-induced cytokine production is downregulated and mice can be protected from death up to 4 hours after a lethal injection of LPS⁴⁵. This is a therapeutic effect that will have obvious implications if it can be reproduced in clinical studies.

Finally, there is the recent description of the monocytic intracellular proteins NOD1 and NOD2 (for nucleotide-binding oligomerization domain), which seem to have the ability to bind and to confer responsiveness to LPS⁴⁶, suggesting that this might be yet another way cells respond to the presence of bacteria⁴⁷. Genotypic variations in *NOD2* are associated with distinct clinical phenotypes of Crohn's disease⁴⁸, prompting speculation that other *NOD* genotypes might be associated with phenotypic variations in LPS responsiveness.

Signal amplification

Following the initial host–microbial interaction there is widespread activation of the innate immune response, the purpose of which is to coordinate a defensive response involving both humoral and cellular components. Mononuclear cells play a key role, releasing the classic pro-inflammatory cytokines IL-1, IL-6 and TNF- α , but in addition an array of other cytokines including IL-12, IL-15 and IL-18, and a host of other small molecules (Table 1).

TNF- α and IL-1 are the prototypic inflammatory cytokines that mediate many of the immunopathological features of LPS-induced shock⁴⁹. They are released during the first 30–90 minutes after exposure to LPS and in turn activate a second level of inflammatory cascades including cytokines, lipid mediators and reactive oxygen species, as well as upregulating cell adhesion molecules that result in the initiation of inflammatory cell migration into tissues. The fact that anti-TNF or anti-IL-1 strategies failed to prevent death in septic patients is probably related more to the difficulty of designing clinical trials in these patients, rather than an intrinsic flaw in the scientific rationale⁵⁰. One practical problem is that patients often come to medical attention relatively late in the disease, and blocking these early cytokines may simply be too late. High mobility group B1

(HMGB1) has recently been identified as a cytokine-like product of macrophages that appears much later after LPS stimulation and may represent a more tractable target for intervention⁵¹.

HMGB1 is a non-histone chromosomal protein that is abundantly distributed and exists in nuclear, cytoplasmic and membrane-bound forms. It participates in stabilizing nucleosomes, facilitates gene transcription and modulates the activity of steroid hormone receptors. When mice were injected with LPS, HMGB1 serum concentrations rose after a delay of about 24 hours, long after the initial peak of IL-1 and TNF- α had declined. Importantly, mice could be rescued from LPS-induced shock by administering an antibody to HMGB1, even when this was provided up to 2 hours after the lethal injection⁵². Subsequently it was shown that patients with sepsis have elevated serum levels of HMGB1, and that higher levels are associated with an increased mortality, suggesting that clinical intervention by blocking or neutralizing HMGB1 might be a viable option.

Another macrophage-derived cytokine that has been identified as a potential therapeutic target in sepsis is macrophage migration inhibitory factor (MIF). Mice with a targeted disruption of the MIF gene are resistant to LPS-induced shock⁵³ and antibody to MIF is fully protective, even in the more demanding caecal ligation and puncture model that resembles clinical peritonitis⁵⁴. MIF also seems to mediate shock caused by Gram-positive bacteria, such as the toxic shock syndrome associated with *S. aureus*⁵⁵, suggesting that anti-MIF strategies might have broad application in septic patients. MIF has a curious relationship with glucocorticoids, which are normally thought of as being anti-inflammatory, as low doses of glucocorticoids paradoxically induce macrophage MIF. Once released, MIF then acts as a pro-inflammatory agent, over-riding the ability of glucocorticoids to prevent shock in animal models of sepsis⁵⁶. How this complex relationship manifests in a clinical setting is of particular interest in the light of the recent studies demonstrating a protective effect of low-dose steroids in patients with severe sepsis.

These pro-inflammatory cytokines are important because they in turn are responsible for orchestrating a complex network of secondary responses (for a review, see ref. 49). A good example of this is provided by IL-18, a cytokine that induces production of interferon- γ (IFN- γ). In human mononuclear cells, IFN- γ upregulates surface expression of TLR4, MD-2 and MyD88, and counteracts the LPS-induced downregulation of TLR4 (ref. 57). It has long been known that IFN- γ sensitizes human mononuclear cells to the effects of LPS, and these new findings suggest strongly that this effect is probably mediated through upregulation (or at least, prevention of downregulation) of TLR4.

The coagulation cascade

Cytokines are also important in inducing a procoagulant effect in sepsis. Disorders of coagulation are common in sepsis, and 30–50% of patients have the more severe clinical form, disseminated intravascular coagulation⁵⁸. Coagulation pathways are initiated by LPS and other microbial components, inducing expression of tissue factor on mononuclear and endothelial cells. Tissue factor in turn activates a series of proteolytic cascades, which result in the conversion of prothrombin to thrombin, which in turn generates fibrin from fibrinogen. Simultaneously, normal regulatory fibrinolytic mechanisms (fibrin breakdown by plasmin) are impaired because of high plasma levels of plasminogen-activator inhibitor type-1 (PAI-1) that prevent the generation of plasmin from the precursor plasminogen. The net result is enhanced production and reduced removal of fibrin leading to the deposition of fibrin clots in small blood vessels, inadequate tissue perfusion and organ failure (Fig. 2).

Pro-inflammatory cytokines, in particular IL-1 and IL-6, are powerful inducers of coagulation, and conversely, IL-10 regulates coagulation by inhibiting the expression of tissue factor on monocytes (for a review, see ref. 59). An additional cause of the procoagulant state in sepsis is the downregulation of three naturally occurring anticoagulant proteins — antithrombin, protein C and tissue factor

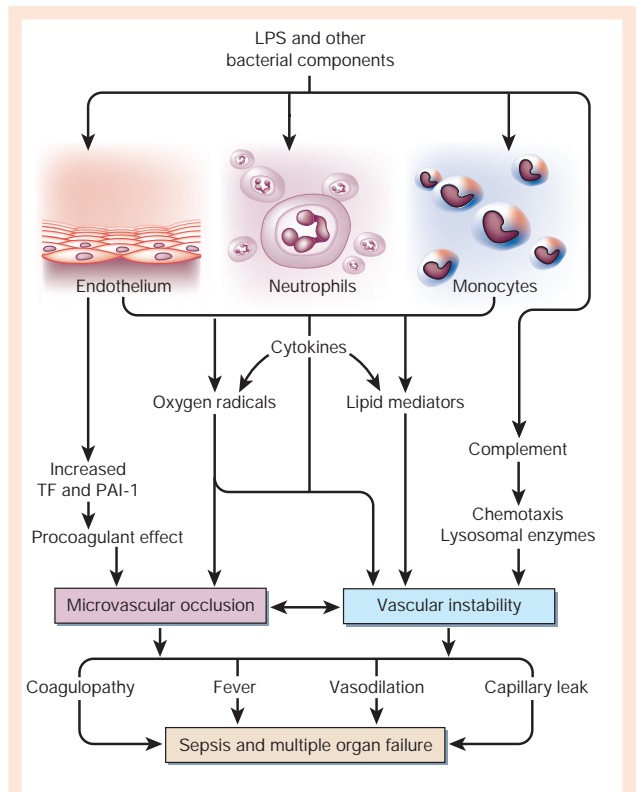


Figure 3 Pathogenetic networks in shock. Lipopolysaccharide (LPS) and other microbial components simultaneously activate multiple parallel cascades that contribute to the pathophysiology of adult respiratory distress syndrome (ARDS) and shock. The combination of poor myocardial contractility, impaired peripheral vascular tone and microvascular occlusion leads to tissue hypoperfusion and inadequate oxygenation, and thus to organ failure.

pathway inhibitor. These natural anticoagulants are of particular interest because in addition to their effect on thrombin generation, they also have anti-inflammatory properties, including effects on release of monocyte-derived TNF- α by inhibiting activation of the transcription factors NF- κ B and activator protein (AP)-1 (ref. 60).

Particular attention has focused on Protein C, which is converted to the activated form (aPC) when thrombin complexes with thrombomodulin, an endothelial transmembrane glycoprotein. Once aPC is formed it dissociates from an endothelial protein C receptor (EPCR) before binding protein S, resulting in inactivation of factors Va and VIIIa and thus blockade of the coagulation cascade. It has been shown recently that aPC uses EPCR as a co-receptor for cleavage of protease-activated receptor 1 (PAR1). Gene profiling showed that PAR1 signalling could account for the activation of aPC-induced protective genes, including the immunomodulatory monocyte chemoattractant protein-1 (MCP-1), suggesting a role for PAR-1 activation in protection from sepsis⁶¹. In septic patients, aPC levels are reduced and expression of endothelial thrombomodulin and EPCR are impaired⁶², providing some support for the notion that replacement of aPC might have therapeutic value.

The counter-inflammatory response — modifier or mediator?

The profound pro-inflammatory response that occurs in sepsis is balanced by an array of counter-regulatory molecules that attempt to restore immunological equilibrium. In this sense, the counter-inflammatory response is seen as a 'modifier' — both appropriate and beneficial. Counter-inflammatory cytokines include antagonists such as the soluble TNF receptors and IL-1 receptor antagonist, decoy receptors such as IL-1 receptor type II, inactivators of the complement

Table 1 Macrophage products implicated in the pathogenesis of sepsis

Mediators	Typical effects
Cytokines	
IL-1, IL-6, IL-12, IL-15, IL-18, TNF- α , MIF, HMGB1, IL-10	Activate neutrophils, lymphocytes and vascular endothelium; upregulate cellular adhesion molecules; induce prostaglandins, nitric oxide synthase and acute-phase proteins; induce fever. Note that IL-10 is predominantly a negative regulator of these effects
Chemokines	
IL-8, MIP-1 α , MIP-1 β , MCP-1, MCP-3	Mobilize and activate inflammatory cells, especially neutrophils; activate macrophages
Lipid mediators	
Platelet-activating factor, prostaglandins, leukotrienes, thromboxane, tissue factor	Activate vascular endothelium; regulate vascular tone; activate extrinsic coagulation cascade
Oxygen radicals	
Superoxide and hydroxyl radicals, nitric oxide	Antimicrobial properties; regulation of vascular tone

Upon activation by microbial components, macrophages release a diverse range of products: the table lists the principal among them that are implicated in the pathogenesis of sepsis. Most of these products have multiple targets and cause effects through several parallel mechanisms. For instance, nitric oxide has direct antimicrobial properties, can modify vascular tone (and hence tissue oxygenation), and also causes macrophage apoptosis. Furthermore, many macrophage products are involved in the regulation of each other. Thus, TNF- α upregulates tissue factor and nitric oxide synthase, IL-18 induces IFN- γ , which in turn further activates macrophages, while IL-10 is a global suppressor of macrophage function. These highly complex and tightly regulated networks make it difficult to predict the consequences of blocking or inhibiting just one pathway.

cascade and the anti-inflammatory cytokines, of which the prototype is IL-10. In concert with this, the host response to injury includes profound changes in metabolic activity (increased cortisol production and release of catecholamines), induction of acute-phase proteins, and endothelial activation with upregulation of adhesion molecules and release of prostanoids and platelet-activating factor (PAF).

Another facet of downregulation of immunity that occurs in sepsis is the development of lymphocyte apoptosis. Extensive lymphocyte apoptosis is seen in animal models of sepsis and is also present in septic patients, although interestingly, much less so in critically ill non-septic controls⁶³. Septic patients are usually lymphopenic, and subset analysis of autopsy tissue samples has shown that there is selective depletion of B and CD4⁺ lymphocytes⁶⁴. This process and its functional consequences are viewed as part of a more general state of immunosuppression, characterized by T-cell hyporesponsiveness and anergy, which occurs to some extent in most septic patients, and which is seen as a counter-balancing response (and sometimes, over-response) to the initial pro-inflammatory state.

It is because of this over-response that some investigators view the counter-inflammatory response as the cause of an inadequate host defence against infection and hence a potential 'mediator' of sepsis and progressive organ failure. Several have pursued the notion that reversal of this immunosuppressive state might be of therapeutic value. For instance, mice transfected with the human gene *bcl-2* that overexpress

the anti-apoptotic protein Bcl-2 are protected from death after caecal ligation and puncture⁶⁵, and patients that received IFN- γ in a small non-randomized clinical study showed upregulation of HLA-DR on their monocytes and a better-than-anticipated survival⁶⁶.

Role of genetic susceptibility in the pathogenesis of sepsis

Among this vast array of host molecules that orchestrate the response to sepsis there are many examples of genetic variability that influence physiological activity. For example, there has been great interest in exploring the possibility that a polymorphism in the TNF promoter that results in significantly higher TNF levels might be associated with a worse outcome from sepsis. Several of these associations have been studied⁶⁷ (Table 2) and at least in some cases the evidence seems convincing. Of particular interest was the recent report that mutations in TLR4 are associated with an increased susceptibility to Gram-negative sepsis³⁶.

Mechanisms of organ failure

The ultimate cause of death in patients with sepsis is multiple organ failure. Typically, patients will first develop a single organ failure — for instance, respiratory failure requiring mechanical ventilation — and then if the disease remains unchecked, will progressively develop failure of other organ systems. There is a close relationship between the severity of organ dysfunction on admission to an intensive care unit and the probability of survival, and between the numbers of organs failing and the risk of death. If four or five organs fail the mortality is greater than 90%, irrespective of treatment.

The pathogenesis of organ dysfunction is multifactorial and incompletely understood. Tissue hypoperfusion and hypoxia are dominant factors (Fig. 3). The mechanisms involve widespread fibrin deposition causing microvascular occlusion, the development of tissue exudates further compromising adequate oxygenation, and disorders of microvascular homeostasis resulting from the elaboration of vasoactive substances such as PAF, histamine and prostanoids. Cellular infiltrates, particularly neutrophils, damage tissue directly by releasing lysosomal enzymes and superoxide-derived free radicals. TNF- α and other cytokines increase the expression of the inducible nitric oxide synthase and increased production of nitric oxide causes further vascular instability and may also contribute to the direct myocardial depression that occurs in sepsis⁶⁸.

The tissue hypoxia that develops in sepsis is reflected in the oxygen debt — that is, the difference between oxygen delivery and oxygen requirements. The extent of the oxygen debt is related to the outcome from sepsis, and strategies designed to optimize oxygen delivery to the tissues can improve survival. In addition to hypoxia, cells may be dysoxic — that is, unable to properly utilize available oxygen. Recent data suggest that this may be another consequence of excess nitric oxide production, because skeletal muscle biopsies from septic

Table 2 Summary of genetic polymorphisms that have been explored in patients with sepsis and meningococcal disease

Host factor	Genetic association	Clinical effect
Tumour-necrosis factor- α (TNF- α)	TNF2 allele G308A mutation in TNF promoter	Increased serum levels of TNF associated with non-survival in sepsis
Lymphotoxin- α (TNF- β)	TNFB2 allele	Increased levels of lymphotoxin associated with sepsis in trauma patients
Interleukin-1 receptor antagonist (IL-1Ra)	ILRN*2 allele	Associated with increased levels IL-1 β and more severe sepsis. Homozygotes for ILRN*2 and TNFB2 have a higher mortality from sepsis
Factor V Leiden	FV ^L	Possibly a mild effect on the severity of meningococcal disease
Tissue plasminogen activator (t-PA)	Insertion/deletion polymorphism	No effect in meningococcal disease
Plasminogen-activator inhibitor 1 (PAI-1)	4G/5G insertion/deletion polymorphism	4G/4G haplotype associated with increased levels of PAI-1 and increased severity of meningococcal disease
FC γ RIIa (CD32)	R131 allotype	More severe outcome from meningococcal disease
Lipopolysaccharide-binding protein (LBP) and bactericidal/permeability-increasing protein (BPI)	Various	No statistically significant effect in sepsis
CD14	C(-159)T promoter polymorphism	Increased susceptibility to septic shock and death
Toll-like receptor 4 (TLR4)	Asp299Gly allele	Significant association with septic shock, and in particular Gram-negative sepsis

It should be noted that not all these associations have been confirmed independently, and the significance of the findings is influenced by the precise patient population studied and the methods used. Other host factors (for instance, mannan binding protein) are known to be important in the response to specific infections, but have not yet been studied specifically in sepsis; yet others (for example, nitric oxide synthase) have been suggested to be important, but clinical data are lacking.

patients show evidence of impaired mitochondrial respiration, which is inhibited by nitric oxide⁶⁹.

Therapeutic approaches

Despite the extraordinary developments in understanding the immunopathology of sepsis, therapeutic advances have been painfully slow. However, in the past 12 months several clinical trials have finally shown that it is possible to reduce mortality in patients with sepsis. Interestingly, each has been based on a different aspect of the pathology described above.

The critical importance of tissue oxygenation was addressed by a study in which patients in the earliest stages of sepsis were treated by aggressive management with fluids, blood transfusion and inotropic agents to optimize haemodynamic function⁷⁰. An alternative approach was taken by van den Bergh and co-workers, who studied the effect of rigorous control of blood glucose levels. Hyperglycaemia and insulin resistance are common in critically ill patients, even if they have not previously had diabetes, and these authors showed that intensive insulin therapy could substantially reduce mortality⁷¹. The mechanism of this striking effect is not absolutely clear, although it is of interest that the greatest reduction in mortality involved deaths due to multiple-organ failure in patients with a proven septic focus, perhaps suggesting that it was related to the better control of the initial infective process.

The third study to show significant benefit was a trial of low-dose corticosteroids. Earlier trials using very high doses of steroids, based on the premise that sepsis represented an uncontrolled inflammatory response, had failed to show any survival benefit. But Annane and colleagues had noted that patients in the advanced stages of septic shock had relative adrenal insufficiency and reasoned that low-dose replacement steroids might be beneficial. In a phase III trial in highly selected patients, they found that low doses of hydrocortisone and fludrocortisone did indeed reduce the mortality substantially⁷². But doubts remain about the precise mechanism of this effect and it is possible that the benefit derives, at least in part, from the immunosuppressive effects of the hydrocortisone.

Disordered coagulation is important in sepsis, and the fourth study examined the effects on the course of sepsis of replacing aPC. A randomized controlled trial of aPC therapy resulted in a significant survival benefit in treated patients⁷³, although interestingly the effect was equally marked in patients whose protein C levels were not depressed. This suggests that the therapeutic effect was attributable in part to the immunosuppressive effects of protein C⁶⁰, and not just to its anticoagulant properties.

Improved understanding of the immunopathology of sepsis has facilitated many other approaches, and several additional strategies are at various stages of development. These include therapies aimed at bacterial targets, for example novel anti-endotoxin molecules such as bactericidal/permeability-increasing protein, or modified lipoproteins, both of which absorb and neutralize LPS, as well as very recent reports that oxidized phospholipids can interfere with binding of LPS to LBP⁷⁴, and strategies aimed at Gram-positive toxins, including competitive antagonists of superantigen-binding sites. There are also investigations aimed at host molecules, such as PAF-receptor antagonists, and a variety of targets in the coagulation cascade. For the clinical investigator, the challenge will be the design of studies with sufficient power to determine the potential value of these new therapies. As fatality rates drop with the use of low-dose steroids, aPC and the other measures described above, it will become increasingly difficult to carry out studies on a heterogeneous population of patients with sepsis. The way forward is likely to lie in identifying clinically relevant endpoints other than death (for instance, reduced incidence of organ failure), and/or identifying more homogeneous subgroups of patients in whom to study specifically targeted therapeutic interventions. □

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