expert reviews
in molecular medicine

Sepsis: molecular mechanisms underlying lipopolysaccharide recognition

Martha Triantafilou and Kathy Triantafilou

Sepsis is an often-fatal response of the immune system against microbial pathogens. The molecular mechanisms that have been designed to protect the host from invading pathogens are responsible for the damage and injury. It is now widely known that this crucial response of the immune system is mediated by innate immunity, which employs a plethora of pattern recognition receptors that recognise motifs expressed by pathogens. A lack of knowledge of the mediators involved in innate recognition has led to unsuccessful attempts at designing effective therapeutic interventions for sepsis. However, in recent years, great leaps forward have been achieved in our knowledge of these mediators. In this review we attempt to unravel the molecular mechanisms underlying bacterial recognition, particularly recognition of bacterial lipopolysaccharide, and we propose future potential therapeutic targets for septic shock.

Bacterial sepsis continues to be the leading cause of death in intensive care units. A large number of patients die as a consequence of an overreaction of the innate immune system to microbial pathogens. The biological processes that are designed to combat the microbial threat become the forces that are responsible for the substantial morbidity and mortality. Annual mortality rates for sepsis are similar to those for myocardial infarction and exceed those of most common cancers (Ref. 1). Existing therapeutic approaches are limited to antibiotic therapy and general supportive care. As our knowledge of the molecular events behind sepsis increases, so do the possible therapeutic interventions.

It is now widely accepted that the overreaction of the host to microbial pathogens occurs at the level of the innate immune system, which is the nonspecific branch of immunity. Thus, the host response is not a result of antigen processing and presentation, or the clonal expansion of cells targeted specifically against the particular

Martha Triantafilou
Senior Research Fellow, University of Sussex, School of Life Sciences, Falmer, Brighton, BN1 9QG, UK. Tel: +44 (0)1273 678923; Fax: +44 (0)1273 678362; E-mail: m.triantafilou@sussex.ac.uk

Kathy Triantafilou (corresponding author)
Reader in Immunology, University of Sussex, School of Life Sciences, Falmer, Brighton, BN1 9QG, UK. Tel: +44 (0)1273 678923; Fax: +44 (0)1273 678362; E-mail: k.triantafilou@sussex.ac.uk

Institute URL: http://www.sussex.ac.uk

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

pathogen, as would occur in the adaptive (acquired) immune system. By contrast, it involves the use of germline-encoded receptors known as pattern recognition receptors (PRRs) that recognise pathogen-associated molecular motifs or patterns (PAMPs) (Ref. 2) encoded only by microbial pathogens and not by the host. PAMPs include components of the bacterial cell wall – such as lipopolysaccharide (LPS) from Gram-negative bacteria, and lipoteichoic acid (LTA) and peptidoglycan from the Gram-positive bacteria – as well as CpG DNA (bacterial DNA rich in cytosine-phosphate diesterguanosine), bacterial flagellin, and double-stranded RNA from viruses (Ref. 3.) Understanding how the innate immune system recognises such diverse microbial products and 'tailors' an inflammatory response against them is a prerequisite to determining how the response can be moderated therapeutically. In this review, we examine recent advances in the field of innate recognition of bacteria, with a primary focus on the molecular events underlying LPS recognition that eventually lead to sepsis.

Role of serum proteins

Bacterial endotoxin or LPS is probably the best-studied bacterial trigger of the innate immune system. Serum proteins such as bactericidal permeability protein (BPI) and LPS-binding protein (LBP) are the first to encounter and bind LPS. Although both of these proteins have been shown to interact with LPS, the outcome of each interaction is completely different. BPI binds and neutralises LPS so that it never reaches its cellular targets (Ref. 4); by contrast, LBP binds to LPS then delivers it to CD14, a 55 kDa cell-surface molecule, in order to activate the innate immune system (Ref. 5).

Other serum proteins have also been shown to be involved in LPS responses. Apolipoprotein binds LPS and reduces production of the pro-inflammatory cytokine tumour necrosis factor α (TNF-α) (Ref. 6). The function of apolipoprotein in LPS responses is further demonstrated by apolipoprotein-knockout mice, which are susceptible to endotoxaemia and sepsis (Ref. 7). Another serum protein that neutralises LPS activity is lactoferrin; lactoferrin inhibits LPS interaction with CD14 by competition with LBP (Ref. 8).

Thus, serum proteins are key molecules in modulating the bioactivity of LPS and in determining whether an inflammatory response will be mounted or not. Some serum proteins have been used as therapeutic targets for sepsis but, in the future, more focus is likely to be placed on designing therapeutic approaches that target the first interactions of LPS with the innate immune system, and this is the focus of this article.

The accepted model of LPS-induced activation

Currently, the accepted model of LPS recognition is based on the seminal discovery that LPS binds LBP in the serum and LPS–LBP complexes in turn bind to CD14 on the cell surface (Ref. 9). CD14 is a receptor expressed primarily on cells of myeloid lineage, such as monocytes and macrophages. Because CD14 is linked to the cell surface by a glycosylphosphatidylnositol (GPI) anchor, rather than by a transmembrane domain, it requires formation of a trimolecular receptor cluster with Toll-like receptor 4 (TLR4) and the accessory protein MD-2 (Refs 10, 11) in order to transduce a signal leading to cell activation. Cell activation is mediated by pathways involving the mitogen-activated protein kinases (MAPKs), including p38 MAPK, c-Jun-N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and the transcription factor NF-κB (Refs 12, 13, 14). This results in an inflammatory response, such as the induction of inflammatory cytokines [interleukin (IL)-1α, IL-6 and TNF-α], that is intended to eradicate the bacterium (Ref. 15) (Fig. 1a).

PRRs involved in LPS responses

The discovery of TLR4 as the signalling receptor for LPS opened the door to a new understanding of innate immunity. The family of ten TLRs (1–10) seem to serve as the central PRRs of the innate immune system (reviewed in Refs 16, 17, 18) and together recognise many different PAMPs (Table 1). Although the general consensus is that the CD14–TLR4–MD-2 complex confers responsiveness to bacterial LPS, several studies have observed CD14-independent binding of LPS (Refs 19, 20, 21, 22). Most importantly, monoclonal antibodies that block CD14 do not inhibit LPS-induced TNF-α secretion, implying that alternative pathways of LPS recognition exist (Ref. 23). Indeed, accumulating evidence is suggesting that the CD14–TLR4–MD-2 model of LPS recognition is an oversimplified one and that several PRRs are involved in LPS responses (Table 1).

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

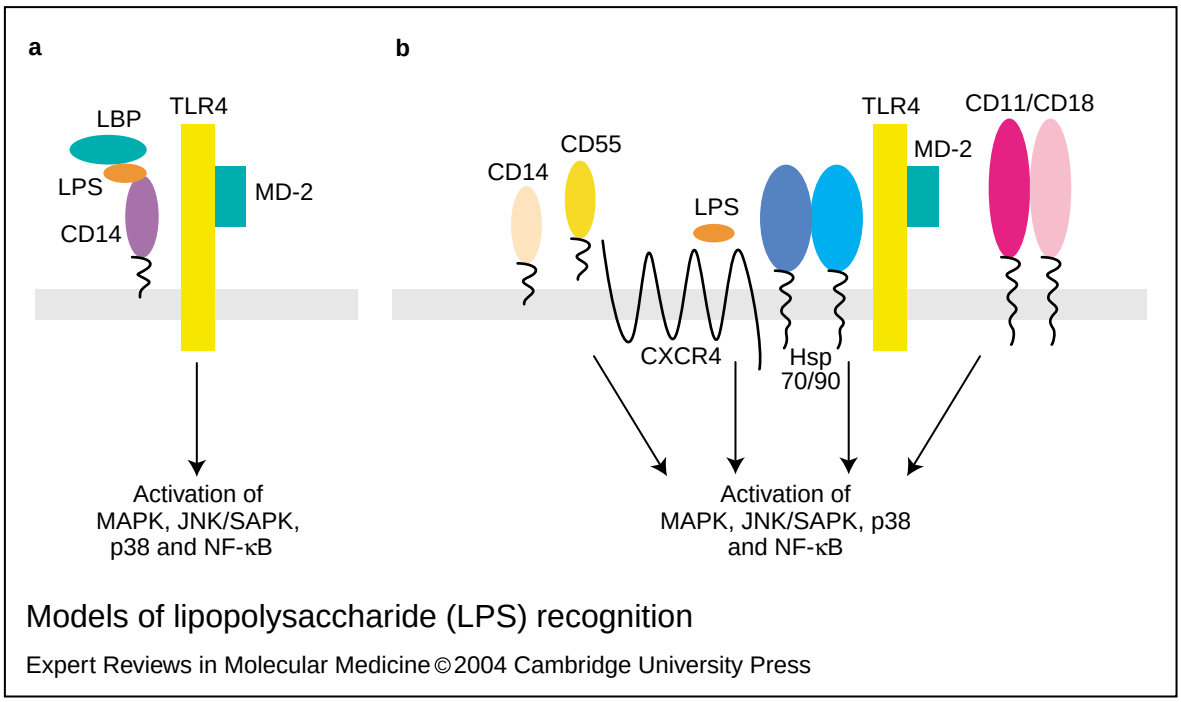


Figure 1. Models of lipopolysaccharide (LPS) recognition. (a) Accepted model of LPS recognition, where LPS binds LPS-binding protein (LBP) in the serum, and then the LPS–LBP complex binds to CD14 on the cell surface of monocytes. CD14 lacks a transmembrane region and cannot transmit a signal; however, following its association with Toll-like receptor 4 (TLR4) and the accessory protein MD-2, signal transduction is triggered, leading to activation of multiple signalling molecules, such as MAPK, JNK/SAPK, p38 and NF-κB. (b) Proposed model of LPS recognition, where LPS binds pattern recognition receptors on the cell surface of monocytes, forming an activation cluster. Multiple signalling molecules are triggered from the multiple receptors that associate within the activation cluster. In addition to CD14, TLR4 and MD-2, other possible interacting molecules are CD55, chemokine receptor 4 (CXCR4), heat shock proteins (Hsps) and the integrin CD11/CD18. However, it might be expected that different combinational associations of receptors occur, enabling the innate immune system to respond to a wide range of microbial pathogens. Abbreviations: JNK/SAPK, c-Jun-N-terminal kinase/stress-activated protein kinase; LBP, LPS-binding protein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa beta (transcription factor); p38, p38 MAPK.

Scavenger receptors

Scavenger receptors, such as CD36, are cell-surface molecules of macrophages that are associated with endocytic uptake of low-density lipoproteins. In addition to this, macrophage scavenger receptors are able to bind LPS and might have an important role in the clearance and detoxification of endotoxin in animals (Ref. 24).

Integrins

The leukocyte integrins CD11b/CD18 and CD11c/CD18 [complement receptor 3 (CR3) and CR4, respectively] are transmembrane, heterodimeric adhesion molecules that interact with a diverse repertoire of ligands including LPS (Refs 25, 26). Like CD14, they are capable of mediating LPS-induced cellular activation (Refs 25, 26). More recently, it has been shown that CD11b/CD18 is involved in cellular activation by LPS and group B streptococci (Refs 27, 28), and also mediates activation of human neutrophils by LPS-coated erythrocytes (Ref. 29).

A mutant CD11b/CD18 that is deficient in its cytoplasmic domain has been shown to be still competent for LPS-induced cellular activation (Ref. 26), and another study has shown that the cytoplasmic domains of integrins are not sufficient for LPS-induced signal transduction (Ref. 30). Thus, it was suggested that integrins do not act alone in LPS recognition. Similarities between the signalling systems utilised by CD14 and CD11/CD18 integrins, such as LBP-dependent binding of whole Gram-negative bacteria and

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

Table 1. Pattern recognition receptors (PAMPs) involved in lipopolysaccharide (LPS) recognition

Receptor	Cell type	Refs
CD14	Monocytes/macrophages	9
TLR4	Most cell types	130
Scavenger receptors	Macrophages	24
CD11b/CD18	Monocytes/macrophages, NK cells, neutrophils	131
CD11c/CD18	Monocytes/macrophages, NK cells, neutrophils, activated lymphocytes	25, 131
CD55	Leukocytes	31
TREM-1	Neutrophils, monocytes	132
RP105 (CD180)–MD1	B cells	37
Hsp70/Hsp90	Most cell types	40, 41
CXCR4	Monocytes, endothelial cells	41
Nod1	All cell types	62
Nod2	All cell types	64

Abbreviations: CXCR4, chemokine receptor 4; Hsp, heat shock protein; NK, natural killer; Nod, nucleotide-binding oligomerisation domain protein; TLR, Toll-like receptor; TREM-1, 'triggering receptor expressed on myeloid cells'.

the same species specificities for LPS analogues (Ref. 30), led to the postulation that integrins, like CD14, might function to bind and transfer LPS to a second signalling molecule.

CD55

Decay accelerating factor (DAF/CD55), which is a regulatory molecule of the complement cascade, has also been implicated in LPS responses: when LPS-hyporesponsive Chinese hamster ovary (CHO) cells were transfected with human CD55, the cells were able to respond to LPS (Ref. 31). Since CD55 is also a GPI-anchored protein, like CD14, it was suggested that it might associate with other signalling molecules in order to transduce the signal, or be part of a multimeric LPS receptor complex (Ref. 32). Recently, Pfeiffer and colleagues have demonstrated that CD55 is indeed part of a complex of receptors, which is concentrated in lipid rafts following LPS stimulation (Ref. 33).

TREM-1

The extensive list of PRRs involved in LPS recognition also includes a new receptor of the immunoglobulin superfamily termed TREM-1 (for 'triggering receptor expressed on myeloid cells'). TREM-1 is expressed on human neutrophils and monocytes, is upregulated by bacterial LPS, and appears to have a predominant role in inflammatory responses (Ref. 34). A recent study demonstrated that use of TREM-1–Ig Fc fusion protein to compete with the unknown TREM-1 ligand resulted in the lowering of serum levels of TNF-α and IL-1β. Most importantly, it was reported that the TREM-1 fusion protein could protect mice from death when given before or after LPS administration (Ref. 35).

RP105

RP105 (CD180) is a type 1 transmembrane protein with extracellular leucine repeats and a short cytoplasmic tail that has also been implicated in

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

LPS responses on B cells (Ref. 36). Expression of RP105 is restricted to mature B cells and macrophages (Ref. 36). The multiple leucine-rich repeats that RP105 possesses are similar to those found in the *Drosophila* Toll protein.

Ogata and colleagues have shown that RP105 regulates LPS signalling on B cells: B cells lacking RP105 were impaired in LPS-induced proliferation and antibody production (Ref. 37). In addition, RP105 has been shown to associate with an extracellular molecule, MD-1 (Ref. 38). RP105–MD-1 is strikingly similar to the TLR4–MD-2 complex in that MD-1 is indispensable for RP105 cell-surface expression as well as B-cell responsiveness to LPS (Ref. 39). Thus, it is thought that RP105–MD-1 constitutes an LPS signalling complex on B cells.

Heat shock proteins

Interestingly, heat shock proteins (Hsps) have recently been implicated in LPS responses. In particular, Hsp70 and Hsp90 have been shown to bind LPS (Refs 40, 41). Hsps are highly conserved proteins that are associated with tissue damage and stress. Although mostly considered as intracellular chaperones in antigen presentation, they have been found to exist on the cell surface (Refs 42, 43, 44, 45) and are emerging as 'danger' signals of the innate immune response (Ref. 46).

Recently, Vabulas and coworkers have shown that specific Hsps interact and activate TLRs, acting as endogenous stimuli for TLRs: Hsp70 activated cells of the innate immune system via the TLR signalling pathway (Ref. 47), whereas Hsp60 as well as Gp96 used TLR2/4 to activate innate immune cells (Refs 48, 49). Furthermore, Hsp internalisation was required in order to stimulate the Toll/IL-1 receptor (TIR) signalling pathway.

In addition, Hsp90 has been shown to bind directly to CpG oligonucleotides (Refs 50, 51), and specific inhibition of Hsp90 using the inhibitor geldanamycin inhibited CpG-induced cellular stimulation (Ref. 50). Hsp90 association with TLR9 was also demonstrated (Ref. 51). Thus, a new role for Hsps in immune responses is emerging. Accumulating evidence is suggesting that Hsps might serve a dual function, bridging both the innate and the adaptive immune responses: one function might be their generally accepted role as chaperones in the antigen-presentation pathway of the adaptive immune response, whereas another function might be to bind and present PAMPs to TLRs as part of the innate immune response. Their recently reported presence in lipid rafts (Refs 52, 53) might facilitate both functions.

Interestingly, recent studies have suggested that LPS contamination of recombinant Hsps might account for the induction of cytokines observed when Hsps are administered (Ref. 54) and for the interactions observed with the 'LPS-sensing machinery' (Ref. 55). Thus, it is possible that Hsps complexed with LPS interact with the TLR pathway and induce CD14-dependent cytokine secretion.

Chemokine receptor 4 (CXCR4)

Chemokine receptor 4 (CXCR4) has also been shown to be involved in LPS responses (Ref. 41). In particular, CXCR4 expression is upregulated after exposure to bacterial products (Ref. 56) and facilitates endothelial cell inflammatory responses. CXCR4 is mainly known as the receptor molecule for human immunodeficiency virus 1 (HIV-1) (Refs 57, 58). Interestingly, LPS has been found to be a potent inhibitor of HIV-1 replication, possibly through downregulation of the CXCR4 receptor (Ref. 59).

Nod proteins

Another class of PRRs, the Nod proteins, has been shown to bind LPS intracellularly and to generate responses against it in the cytosol (Ref. 60). Nod1 and Nod2 are composed of an N-terminal caspase recruitment domain, a centrally located nucleotide domain, and a C-terminal domain of leucine-rich repeats (Ref. 60). They are thought to bind bacterial products such as LPS and peptidoglycan through the leucine-rich repeats, in a similar manner to CD14 and TLR4 (Ref. 61). Nod1 and Nod2 detect similar yet distinct muropeptides: Nod1 senses a unique diaminopimelate-containing *N*-acetylglucosamine-*N*-acetylmuramic acid (GlcNAc-MurNAc) tripeptide found in Gram-negative bacterial peptidoglycan (Refs 62, 63), whereas Nod2 detects GlcNAc-MurNAc-dipeptide (Refs 63, 64).

In response to bacterial products, Nod proteins are able to activate the NF-κB pathway (Ref. 65), which plays a major role in innate immunity. Most interestingly, a mutation in the coding region of Nod2 has been associated with susceptibility to Crohn's disease (Refs 66, 67), a chronic inflammatory disorder of the intestinal track. This mutation seems to render Nod2 incapable of

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

triggering NF-κB activation in response to LPS. Thus, the family of Nod proteins are emerging as intracellular PRRs that are responsible for sensing bacteria and bacterial products inside the cell.

Activation clusters

The discovery of many molecules implicated in LPS responses suggests that the CD14–TLR4–MD-2 model for LPS recognition is a simplified one. Although these three core molecules are involved in the recognition of LPS, different combinations of additional receptors in 'activation clusters' might be involved depending on the ligand and on the cell type (Fig. 1b). Thus, different combinational associations of receptors enable the innate immune system to respond to a wide range of microbial pathogens and to 'tailor' the inflammatory response against that particular microbial invader.

Evidence for the formation of activation clusters

Biochemical and fluorescent imaging methods have demonstrated the formation of an activation cluster following LPS stimulation that comprises Hsp70, Hsp90, CXCR4 and growth differentiation factor 5 (GDF5) (Ref. 41). Incubation with antibodies against the receptor proteins of the cluster abrogated TNF-α secretion.

The formation of different activation clusters has also been observed in response to LPS, LTA and ceramide (Ref. 33). A receptor cluster comprising CD11b/CD18, CD14, CD55, CD81, Fcγ-R CD16a, scavenger receptor CD36 and TLR4 was formed following LPS stimulation. Stimulation with LTA induced a similar receptor cluster, but, interestingly, the components of the activation cluster changed when cells were stimulated with ceramide. This suggests that different combinational associations of receptors might underlie the recognition of a wide range of microbial stimuli by the innate immune system. Similarly, recent experiments have shown that different activation clusters are formed following stimulation with different LPS analogues, such as pentaacyl lipid A and lipid Via (M. Triantafilou et al., unpublished). TLR4 does not seem to be recruited in these clusters, which could explain why there is no cellular activation following stimulation by LPS antagonists.

Another study has identified CD55 as a receptor that binds LPS and is part of the LPS activation cluster (Ref. 32). In addition, the interaction of CD14, CD11b/CD18 and TLR4 is required for LPS- and taxol-inducible gene expression (Ref. 68), further strengthening the idea of activation clusters as part of innate immunity.

In addition to showing differential association with different receptor molecules, different TLRs can co-operate in response to microbial pathogens. For example, TLR2 seems to form functional pairs with either TLR1 or TLR6: TLR2-mediated cytokine responses were enhanced by TLR6 but inhibited by TLR1 (Refs 69, 70). These findings strengthen the concept of different activation clusters being formed as part of the innate immune response.

Microdomains

In a similar manner to the adaptive immune response (Ref. 71), the innate immune system seems to require 'partitioning' of the plasma membrane for transduction of the activation signal in response to LPS. Lipid rafts (or microdomains) are defined as dynamic assemblies of lipids and specific proteins in the biological membrane. One of the functions of lipid rafts is the recruitment and concentration of molecules involved in cellular signalling (Ref. 72). Lipid rafts are believed to be sites for the compartmentalisation of signalling events.

It has been previously shown that CD14 resides within lipid rafts (Ref. 73), and recently formation of activation clusters comprising CD14, Hsp70, Hsp90, CXCR4 and TLR4 were demonstrated in response to LPS within lipid rafts (Ref. 52). This compartmentalisation aids in LPS-induced signalling, since raft-disrupting drugs inhibit LPS-induced cytokine production (Ref. 52). It seems that, upon ligation of CD14 by LPS, recruitment of different receptors and TLR4 occurs at the site of ligation within lipid rafts, and the signalling cascade is triggered.

Lipid rafts also seem to be important for LPS internalisation. LPS rapidly internalises, along with CD14 and TLR4, through a lipid-raft-dependent pathway and is targeted to the Golgi complex (Ref. 74).

Sepsis: molecular mechanisms of onset

Sepsis is a clinical disorder that is caused by the host's response to infection. The disease is paradoxical, since the host immune response that has been designed to protect against a hostile microbial environment is the cause of injury and damage to the host. Recently, investigators

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

have suggested that sepsis might not be attributed solely to the inflammatory response but perhaps also to a compromised immune system that is unable to eradicate pathogens (Ref. 75).

The onset of sepsis seems to result from a combination of uncontrolled cascades of coagulation, fibrinolysis and inflammation. Following bacterial infection and triggering of the host's inflammatory response, the production of pro-inflammatory cytokines, such as IL-1α, IL-1β and TNF-α, have direct damaging actions on the vascular endothelium (Ref. 76). One of these damaging actions is the biosynthesis of lipid mediators through the action of enzymes of the phospholipase A_2 (PLA_2) family. PLA_2 catalyses the conversion of cell membrane phospholipids to platelet-activating factor (PAF) (Ref. 77). Release of PAF and engagement of its cellular receptor activates multiple cascades that contribute to the acute inflammatory injury (Ref. 78).

One of the most damaging actions triggered by the production of pro-inflammatory cytokines is the initiation of coagulation. In sepsis, the production of pro-inflammatory cytokines induces the expression of tissue factor (TF) on endothelial cells as well as monocytes. The expression of TF triggers the extrinsic coagulation cascade (Ref. 79). TF interacts with factor VIIa, forming a complex that in turn activates factors X and IX. This activation leads to the activation of factors XI, VII and V, which results in the generation of large amounts of thrombin (Fig. 2). Under normal circumstances, endogenous modulators of homeostasis, such as protein C and antithrombin III, would modulate this cascade. In sepsis, these modulators are consumed as the immune system attempts to return to a normal state. Since the coagulation cascade remains uncontrolled, it results in widespread coagulopathy and microvascular thrombosis. Disseminated intravascular coagulation (DIC) is a late complication of sepsis that leads to multiple organ failure (Ref. 80) and ultimately death for a large number of patients.

Clinical and therapeutic implications

The hope of averting approximately 200 000 deaths annually in the USA alone from sepsis (Ref. 1) has sparked great interest in the search for therapeutic interventions for sepsis (Table 2). However, at best, efforts to achieve this have been disappointing and current therapeutic interventions are still restricted to antibiotics and general supportive care. This section summarises the different therapeutic strategies that have been attempted, in an effort to learn from their outcome and inform future attempts.

Therapeutic interventions attempted for septic shock

One attempt to inhibit the effects of LPS has been to use antibodies against the endotoxin itself. Two different monoclonal antibodies against LPS have been used in multicentre clinical trials (Ref. 81): HA-1A, a human monoclonal antibody; and E5, a murine antibody. The antibodies had little or no effect on a 28-day mortality rate. If anything, there was increased mortality for patients who did not have endotoxaemia (Ref. 81). The use of polyvalent antibodies pooled from several donors has also been explored (Ref. 82). Although utility was tested in several small studies, the data showed a significant reduction in mortality (Ref. 82).

Another avenue that has been explored is to attempt to target inflammatory cytokines. Two strategies have been used to neutralise TNF-α: one used a soluble TNF receptor; the other used a monoclonal antibody against TNF-α. Two receptors have been identified for TNF: TNFRI (p55) and TNFRII (p75) (Ref. 83). Fusion proteins consisting of the extracellular domain from both receptors fused to the Fc portion of an immunoglobulin were generated and used in clinical trials. Although these fusion proteins showed encouraging results in Phase II clinical trials, they failed to show a significant reduction in mortality in Phase III (Ref. 84). Monoclonal antibodies against TNF-α proved to be more valuable. Ten multicentre, randomised trials have been conducted with various anti-TNF antibodies. They have all shown a statistically significant 3.5% reduction in mortality (Refs 85, 86, 87, 88, 89, 90, 91). The cytokine IL-1 has also been targeted. Recombinant IL-1 receptor antagonist (IL-1ra) has been used in three different clinical trials. Pooled data from the three studies showed a 4.9% reduction in mortality (Refs 92, 93).

Human serum proteins have also been used to neutralise LPS in the serum. Recombinant BPI (rBPI) was used in a trial with children suffering from meningococcaemia. A modest reduction in mortality was observed (Ref. 94). A neutrophil granule protein, CAP18 ('cationic antimicrobial protein 18 kDa'), has also been used in preclinical studies, with promising results (Ref. 95). Other

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

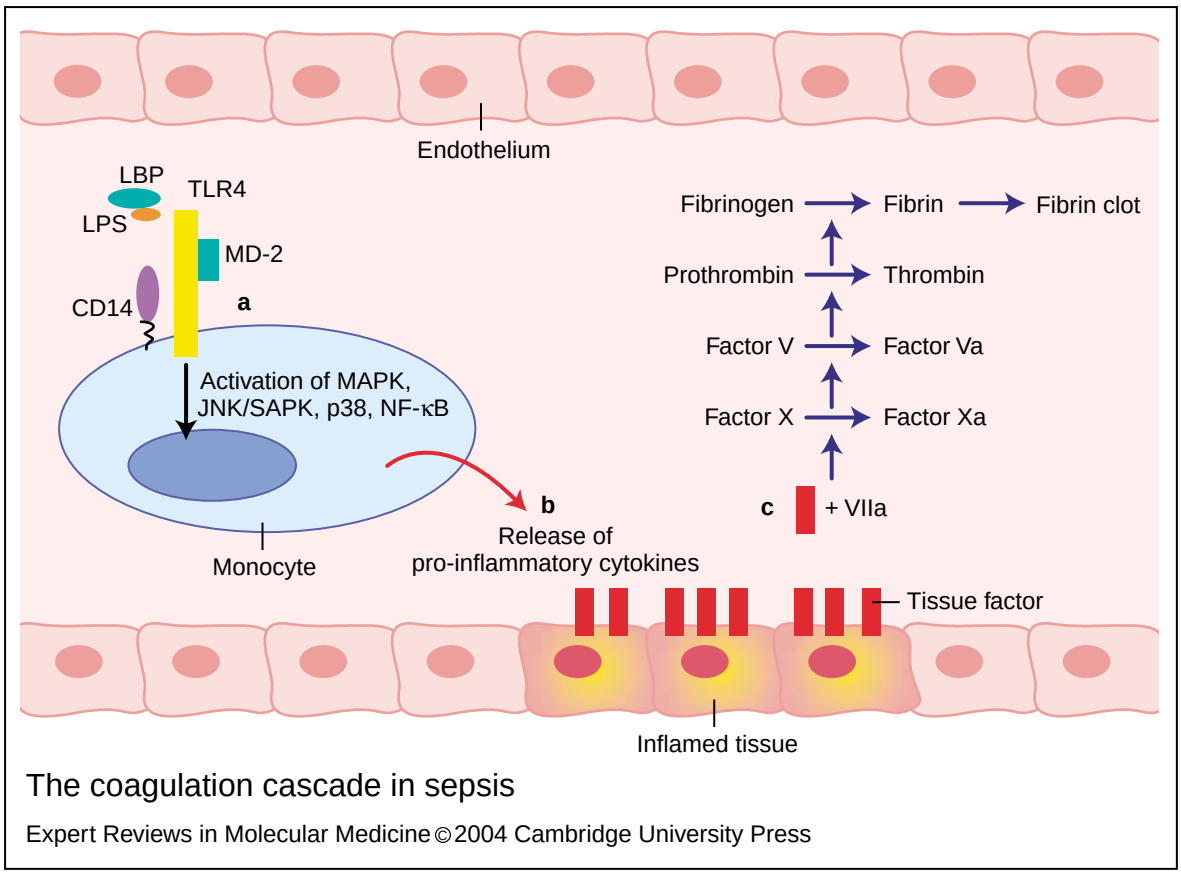


Figure 2. The coagulation cascade in sepsis. (a) In the blood stream, complexes of lipopolysaccharide (LPS) and LPS-binding protein (LBP) bind to their receptors on the cell surface of monocytes, triggering the production of pro-inflammatory cytokines. (b) Production of pro-inflammatory cytokines induces the expression of tissue factor on endothelial cells, (c) which in turn interacts with factor VIIa to initiate the coagulation cascade (factor VIIa catalyses the conversion of factor X to factor Xa, leading to activation of factor V, conversion of prothrombin to thrombin, and subsequent conversion of fibrinogen to fibrin and clots). Abbreviations: JNK/SAPK, c-Jun-N-terminal kinase/stress-activated protein kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa beta (transcription factor); p38, p38 MAPK; TLR, Toll-like receptor.

strategies have included the administration of high-density lipoprotein (Ref. 96), and the antibiotic polymyxin B (which binds and chelates endotoxin) (Ref. 97)

PLA_2 is a family of enzymes that have been shown to be elevated in patients with sepsis (Ref. 98). Clinical trials with inhibitors of PLA_2 have been conducted. A Phase II clinical trial with PLA_2 inhibitor (Ly315920) showed no benefit for patients with sepsis (Ref. 99). PLA_2 stimulates the generation of PAF from membrane phospholipids, which stimulates the production of proinflammatory cytokines. Two strategies have been used to combat PAF: one strategy attempts to block PAF binding to its receptor by utilising synthetic antagonists; the other employs the activity of PAF acetylhydrolase, an enzyme that inactivates PAF. The synthetic antagonists used were BN52021 (Ginkgolide B), TCV-309 and BB-882 (Lexipafant). BN52021 and TCV-309 showed benefit in clinical trials, whereas Lexipafant showed no apparent benefit (Refs 100, 101, 102). The second strategy, administering PAF acetylhydrolase, resulted in significant improvement in a 28-day mortality trial in an unpublished Phase II study. However, a Phase III clinical trial was discontinued since it showed no benefit (Ref. 103).

Nitric oxide (NO) is another mediator that contributes to the injury of sepsis (Ref. 104). Nonspecific inhibitors of NO such as the methylated arginine analogues L-*N*-monomethyl

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

Table 2. Therapeutic interventions for septic shock

Therapeutic target	Drug used	Outcome	Refs
Endotoxin	HA-1A human mAb	Little or no clinical benefit	81
	E5 murine antibody	Little or no clinical benefit	81
	Polyvalent antibodies	Significant mortality benefit	82
	LPS antagonist (E5531)	Reduction in mortality in a Phase II trial	–[a]
Inflammatory cytokines	Fusion proteins of TNFRI (p55) and TNFRII (p75)	No clinical benefit	83, 84
	mAb against TNF-α	3.5% reduction in mortality	85, 86, 87, 88, 89, 90, 91
	Recombinant IL-1ra	4.9% reduction in mortality	92, 93
Serum proteins	Recombinant BPI	Modest reduction in mortality	94
	CAP18	No clinical benefit	95
Lipid mediators	PLA_2 inhibitor (Ly315920)	No clinical benefit	99
	Synthetic antagonists of PAF [BN52021 (Ginkgolide B) and TCV-309]	Some clinical benefit	100, 102
	Synthetic antagonist of PAF [BB-882 (Lexipafant)]	No clinical benefit	101
	PAF acetylhydrolase	No clinical benefit	103
NO	Inhibitors of NO such as methylated arginine analogues L-NMMA and L-NAME	No clinical benefit	123
Immunostimulatory molecules	IFN-γ	No clinical benefit	123
	G-CSF	No clinical benefit	105
Coagulation	Protein C concentrate	Great reduction in mortality	108
	Antithrombin	No clinical benefit	109
	TFPI	Reduced mortality in a Phase II trial	110
	Site inhibition of factor VIIa	Blocks thrombin and fibrin	111
Inflammation	Large doses of corticosteroids	No clinical benefit	113, 114
	Low doses of corticosteroids	Significant reduction in mortality	115

[a] http://www.eisai.com

Abbreviations: BPI, bactericidal permeability protein; CAP18, cationic antimicrobial protein 18 kDa; IL-1ra, interleukin 1 receptor antagonist; G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon γ; LPS, lipopolysaccharide; mAb, monoclonal antibody; NO, nitric oxide; L-NAME, *N*-omega-nitro-L-arginine methyl ester; L-NMMA, L-*N*-monomethyl arginine; PLA_2, phospholipase A_2; TFPI, tissue factor pathway inhibitor; TNFR, tumour necrosis factor receptor.

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

arginine (L-NMMA) and *N*-omega-nitro-L-arginine methyl ester (L-NAME) were used in several small studies. They resulted in an increase in blood pressure, which might in part have contributed to the increased mortality rate observed in a Phase III clinical trial of L-NMMA that was aborted (Ref. 104).

Administration of the immunostimulators interferon-γ (IFN-γ) or granulocyte colony-stimulating factor (G-CSF) has also been tried. The clinical benefit of IFN-γ remains to be proven; similarly, although G-CSF has anti-inflammatory activity, a large multicentre trial showed no clinical benefit (Ref. 105).

Other strategies have targeted the coagulation pathway in sepsis. In healthy individuals, coagulation is controlled by the protein C pathway, the antithrombin pathway and the TF pathway inhibitor (TFPI) (Ref. 106). In sepsis, all these pathways are disturbed, leading to problems with coagulation. Protein C concentrate has been evaluated in several small studies with children with meningococcaemia (Ref. 107); a multicentre Phase III clinical trial showed a great reduction in 28-day mortality (Ref. 108). By contrast, an international multicentre Phase III clinical trial of antithrombin failed to show any clinical benefit (Ref. 109). TFPI reduced mortality in a Phase II trial (Ref. 110) but showed no clinical benefit in a Phase III trial. In addition, direct inhibition of TF by antibody or the antagonism of factor VIIa and Xa has also been tried. Site inhibition of factor VIIa was found to block thrombin and fibrin production (Ref. 111), whereas there are no results available for a small-molecule inhibitor of factor Xa.

Corticosteroids have long been thought of as an attractive intervention for sepsis because of their potent anti-inflammatory activity. Administration of large doses of corticosteroids was initially thought to improve patient survival for sepsis (Ref. 112), but later studies with high-dose corticosteroids suggested that there was no clinical benefit (Ref. 113) and this conclusion was supported by a meta-analysis of all the trials (Ref. 114). Recently, the possibility of administrating low doses of corticosteroids was explored. In a randomised, multicentre trial, a significant reduction in mortality was observed (Ref. 115).

Lipid As from nontoxic bacteria such as *Rhodobacter capsulatus* or *Rhodobacter sphaeroides*, as well as their synthetic analogues, have been shown to antagonise the effects of lipid A from pathogenic bacteria (Refs 116, 117). Thus, one concept for therapeutic intervention in sepsis is the application of endotoxin antagonists (Refs 116, 118, 119, 120). The tetraacyl compound 406 (also called lipid IVa) was the first synthetic endotoxin structure reported with antagonistic properties (Ref. 120). More recently, a synthetic analogue termed E5531 was found to be able to inhibit cellular activation in response to LPS (Refs 121, 122). Eisai Co., Ltd of Tokyo is currently in Phase II clinical trials with E5531 for septic shock (http://www.eisai.com).

It is evident from the cataloguing of all clinical trials attempted (Ref. 123) that only a handful have demonstrated a small reduction in mortality. Only two mediators seem to have a good clinical effect: activated protein C has made it to the market and hydrocortisone, a corticosteroid, has found a new clinical application. Thus, the search for therapeutic interventions for septic shock continues, with the hope that one of the newly identified mediators will prove effective and the significant number of fatalities can be reduced.

Designing more-effective therapeutic interventions

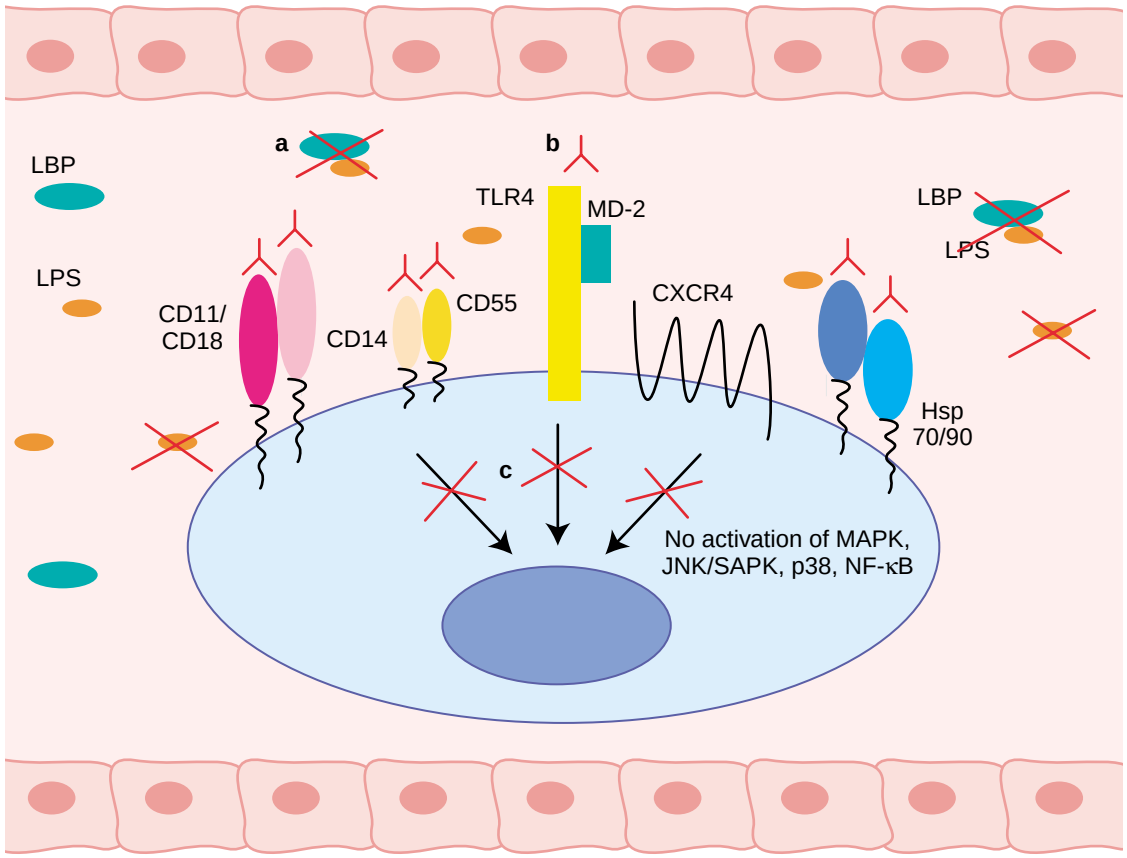
As described here, numerous PRRs are involved in the recognition of microbial products and the generation of an inflammatory response against them. The lack of successful therapeutic targets is a direct reflection of our lack of understanding of the mechanisms behind innate recognition that subsequently lead to sepsis. However, in the past few years, major breakthroughs have been achieved in knowledge of innate recognition of LPS, and many other molecules have been found to be involved, including CD14, TLR4, scavenger receptors, integrins, CD55, TREM-1, CXCR4 and heat shock proteins. Accumulating evidence is suggesting that different combinational associations of these receptors occur in response to different ligands, thereby allowing innate immunity to recognise a wide range of microbial pathogens. Although we know that these clusters are involved in LPS recognition, we still do not completely understand how all of these membrane proteins fit together in the 'LPS-sensing' apparatus of the innate immune system. Once this has been deciphered, we should be able to design more-effective therapeutic interventions. In the meantime, we have to attempt to guess how we can best solve this puzzle.

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

The targets that we can use in order to modulate the host inflammatory response in sepsis are numerous and diverse. As mentioned above, researchers have already targeted many with varying success. Others not yet targeted include cell-surface receptors for endotoxin, such as the PRRs mentioned earlier, and the intracellular signalling cascades that they trigger.

An important consideration in the design of an effective therapeutic intervention is the timing of the intervention (Fig. 3). If the patient is in the early phases of endotoxaemia, the ideal intervention would be to neutralise LPS in the serum before it reaches its cellular targets, or to block LPS receptors such as CD14, TLR4 and other PRRs. Targeting multiple receptors at the same time should prove to be more efficient given the role of activation clusters in LPS-induced cytokine production. Cocktails of blocking monoclonal antibodies against these receptors could be used.

At later stages of endotoxaemia, inhibitors of the multiple signalling cascades that are triggered



Potential therapeutic targets for sepsis

Figure 3. Potential therapeutic targets for sepsis. In the blood stream, lipopolysaccharide (LPS) binds LPS-binding protein (LBP) and then is proposed to bind to an activation cluster on the surface of monocytes. In the early stages of endotoxaemia, a potential therapeutic approach could be: (a) to neutralise LPS in the serum before it reaches its cellular targets; or (b) to block the pattern recognition receptors (PRRs) that bind LPS using a cocktail of monoclonal antibodies. (c) In later stages of endotoxaemia, simultaneous inhibition of multiple signalling cascades might prove effective. Abbreviations: CXCR4, chemokine receptor 4; Hsp, heat shock protein; JNK/SAPK, c-Jun-N-terminal kinase/stress-activated protein kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa beta (transcription factor); p38, p38 MAPK; TLR, Toll-like receptor.

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

could be utilised. In a recent in vitro study analysing the signalling cascades triggered by group B streptococci, it was shown that simultaneous activation of multiple pathways, including NF-κB and p38 MAPKs, is required to induce maximal TNF-α production (Ref. 124). Selective inhibitors of MAPK (PD98059 or U0126), p38 (SB203580) or NF-κB (caffeic acid phenetyl ester) could reduce TNF-α production. Although none of the inhibitors used alone could totally inhibit TNF-α production (Ref. 124), combinations of the inhibitors might totally inhibit cytokine production. This study is the perfect example of targeting multiple mediators at the same time. Since the mediators involved in sepsis are so numerous and diverse, only an approach involving multiple targets is likely to prove effective.

The TLR signalling cascade is also an obvious therapeutic target. All TLRs display a conserved cytoplasmic motif, the TIR domain (Ref. 125). This domain was thought to interact with the adaptor molecule MyD88, which acted as a transducer for all TLRs. However, recently MyD88 was shown not to be the sole adaptor for TLR signalling, since mice lacking MyD88 still exhibited some LPS responses (Ref. 126). Thus, TLRs were shown to trigger both MyD88-dependent and -independent signalling cascades (Ref. 126). TIRAP (also designated Mal for 'MyD88-adapter-like') (Ref. 127) was thought to serve the MyD88-independent pathway, but genetic evidence revealed that TIRAP and MyD88 act in conjunction (Ref. 128). Recently, it was revealed that the TIR adaptor protein Trif is the key transducer of MyD88-independent signalling (Ref. 129). If both the Trif and MyD88 pathways are targeted at the same time then TLR signalling should be completely incapacitated.

Concluding remarks

Research into the innate recognition of bacteria has reached a stage where many of the molecules involved have been identified. The challenge has now changed from identifying the mediators to determining how all these molecules fit together. As our knowledge increases, so will possibilities for specific targeting of crucial components of the LPS-sensing apparatus. Is finding a single therapeutic intervention for sepsis the 'holy grail' of the innate immune research? In our opinion, it is not. However, if multiple receptors and multiple signalling cascades are tageted, we should be able to put a stop to this lethal overreaction of the innate immune system.

Acknowledgements and funding

We thank our three anonymous peer reviewers for their comments. Our current research is supported by the BBSRC, Wellcome Trust and Sport Aiding Medical Research for Kids (SPARKs).

References

1 Angus, D.C. et al. (2001) Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 29, 1303-1310, PubMed: 11445675
2 Medzhitov, R. and Janeway, C.A., Jr. (1998) An ancient system of host defense. Curr Opin Immunol 10, 12-15, PubMed: 9523104
3 Alexopoulou, L. et al. (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732-738, PubMed: 11607032
4 Elsbach, P. and Weiss, J. (1993) The bactericidal/permeability-increasing protein (BPI), a potent element in host-defense against gram-negative bacteria and lipopolysaccharide. Immunobiology 187, 417-429, PubMed: 8330906
5 Tobias, P.S. et al. (1997) Lipopolysaccharide (LPS)-binding proteins BPI and LBP form different types of complexes with LPS. J Biol Chem 272, 18682-18685, PubMed: 9228038
6 Hubsch, A.P. et al. (1993) A reconstituted, apolipoprotein A-I containing lipoprotein reduces tumor necrosis factor release and attenuates shock in endotoxemic rabbits. Circ Shock 40, 14-23, PubMed: 8324886
7 de Bont, N. et al. (1999) Apolipoprotein E knock-out mice are highly susceptible to endotoxemia and Klebsiella pneumoniae infection. J Lipid Res 40, 680-685, PubMed: 10191292
8 Elass-Rochard, E. et al. (1998) Lactoferrin inhibits the endotoxin interaction with CD14 by competition with the lipopolysaccharide-binding protein. Infect Immun 66, 486-491, PubMed: 9453600
9 Wright, S.D. et al. (1990) CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 249, 1431-1433, PubMed: 1698311
10 Shimazu, R. et al. (1999) MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med 189, 1777-1782,

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

PubMed: 10359581
11 Akashi, S. et al. (2000) Cutting edge: cell surface expression and lipopolysaccharide signaling via the toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages. J Immunol 164, 3471-3475, PubMed: 10725698
12 Weinstein, S.L., Gold, M.R. and DeFranco, A.L. (1991) Bacterial lipopolysaccharide stimulates protein tyrosine phosphorylation in macrophages. Proc Natl Acad Sci U S A 88, 4148-4152, PubMed: 1709735
13 Hambleton, J. et al. (1996) Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages. Proc Natl Acad Sci U S A 93, 2774-2778, PubMed: 8610116
14 Swantek, J.L., Cobb, M.H. and Geppert, T.D. (1997) Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor alpha (TNF-alpha) translation: glucocorticoids inhibit TNF-alpha translation by blocking JNK/SAPK. Mol Cell Biol 17, 6274-6282, PubMed: 9343388
15 O'Neill, L. (2000) The Toll/interleukin-1 receptor domain: a molecular switch for inflammation and host defence. Biochem Soc Trans 28, 557-563, PubMed: 11044374
16 Medzhitov, R. (2001) Toll-like receptors and innate immunity. Nat Rev Immunol 1, 135-145, PubMed: 11905821
17 Cario, E. (2003) Toll-like receptors and intestinal defence: molecular basis and therapeutic implications. Expert Rev Mol Med 5, 1-15, PubMed: 14585165
18 Akira, S. (2001) Toll-like receptors and innate immunity. Adv Immunol 78, 1-56, PubMed: 11432202
19 Lynn, W.A., Liu, Y. and Golenbock, D.T. (1993) Neither CD14 nor serum is absolutely necessary for activation of mononuclear phagocytes by bacterial lipopolysaccharide. Infect Immun 61, 4452-4461, PubMed: 7691750
20 Blondin, C. et al. (1997) Lipopolysaccharide complexed with soluble CD14 binds to normal human monocytes. Eur J Immunol 27, 3303-3309, PubMed: 9464818
21 Troelstra, A. et al. (1997) Saturable CD14-dependent binding of fluorescein-labeled lipopolysaccharide to human monocytes. Infect Immun 65, 2272-2277, PubMed: 9169763
22 Triantafilou, M., Triantafilou, K. and Fernandez, N. (2000) Rough and smooth forms of fluorescein-labelled bacterial endotoxin exhibit CD14/LBP dependent and independent binding that is influencedby endotoxin concentration. Eur J Biochem 267, 2218-2226, PubMed: 10759844
23 Gessani, S. et al. (1993) Enhanced production of LPS-induced cytokines during differentiation of human monocytes to macrophages. Role of LPS receptors. J Immunol 151, 3758-3766, PubMed: 7690813
24 Hampton, R.Y. et al. (1991) Recognition and plasma clearance of endotoxin by scavenger receptors. Nature 352, 342-344, PubMed: 1852209
25 Ingalls, R.R. and Golenbock, D.T. (1995) CD11c/CD18, a transmembrane signaling receptor for lipopolysaccharide. J Exp Med 181, 1473-1479, PubMed: 7535339
26 Ingalls, R.R., Arnaout, M.A. and Golenbock, D.T. (1997) Outside-in signaling by lipopolysaccharide through a tailless integrin. J Immunol 159, 433-438, PubMed: 9200483
27 Medvedev, A.E. et al. (1998) Involvement of CD14 and complement receptors CR3 and CR4 in nuclear factor-kappaB activation and TNF production induced by lipopolysaccharide and group B streptococcal cell walls. J Immunol 160, 4535-4542, PubMed: 9574560
28 Chow, J.C. et al. (1999) Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem 274, 10689-10692, PubMed: 10196138
29 Troelstra, A. et al. (1999) Lipopolysaccharide-coated erythrocytes activate human neutrophils via CD14 while subsequent binding is through CD11b/CD18. J Immunol 162, 4220-4225, PubMed: 10201950
30 Ingalls, R.R. et al. (1998) CD11/CD18 and CD14 share a common lipid A signaling pathway. J Immunol 161, 5413-5420, PubMed: 9820516
31 Heine, H. et al. (2001) Decay-accelerating factor (DAF/CD55) is a functional active element of the LPS receptor complex. J Endotoxin Res 7, 227-231, PubMed: 11581575
32 Heine, H. et al. (2003) CD55/decay accelerating factor is part of the lipopolysaccharide-induced receptor complex. Eur J Immunol 33, 1399-1408, PubMed: 12731067
33 Pfeiffer, A. et al. (2001) Lipopolysaccharide and ceramide docking to CD14 provokes ligand-specific receptor clustering in rafts. Eur J Immunol 31, 3153-3164, PubMed: 11745332
34 Bouchon, A., Dietrich, J. and Colonna, M. (2000) Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 164,

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

4991-4995, PubMed: 10799849
35 Bouchon, A. et al. (2001) TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 410, 1103-1107, PubMed: 11323674
36 Miyake, K. et al. (1995) RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the leucine-rich repeat protein family. J Immunol 154, 3333-3340, PubMed: 7897216
37 Ogata, H. et al. (2000) The toll-like receptor protein RP105 regulates lipopolysaccharide signaling in B cells. J Exp Med 192, 23-29, PubMed: 10880523
38 Miyake, K. et al. (2000) Innate recognition of lipopolysaccharide by Toll-like receptor 4/MD-2 and RP105/MD-1. J Endotoxin Res 6, 389-391, PubMed: 11521060
39 Nagai, Y. et al. (2002) Requirement for MD-1 in cell surface expression of RP105/CD180 and B-cell responsiveness to lipopolysaccharide. Blood 99, 1699-1705, PubMed: 11861286
40 Byrd, C.A. et al. (1999) Heat shock protein 90 mediates macrophage activation by Taxol and bacterial lipopolysaccharide. Proc Natl Acad Sci U S A 96, 5645-5650, PubMed: 10318938
41 Triantafilou, K., Triantafilou, M. and Dedrick, R.L. (2001) A CD14-independent LPS receptor cluster. Nat Immunol 2, 338-345, PubMed: 11276205
42 Kaur, I. et al. (1993) Human peripheral gamma delta T cells recognize hsp60 molecules on Daudi Burkitt's lymphoma cells. J Immunol 150, 2046-2055, PubMed: 8094731
43 Multhoff, G. et al. (1997) Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells. J Immunol 158, 4341-4350, PubMed: 9126997
44 Tsuboi, N. et al. (1994) Monoclonal antibody specifically reacting against 73-kilodalton heat shock cognate protein: possible expression on mammalian cell surface. Hybridoma 13, 373-381, PubMed: 7860094
45 Tamura, Y. et al. (1993) 70 kDa heat shock cognate protein is a transformation-associated antigen and a possible target for the host's anti-tumor immunity. J Immunol 151, 5516-5524, PubMed: 8228242
46 Todryk, S.M. et al. (2000) Heat shock proteins refine the danger theory. Immunology 99, 334-337, PubMed: 10712661
47 Vabulas, R.M. et al. (2002) HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. J Biol Chem 277, 15107-15112, PubMed: 11842086
48 Vabulas, R.M. et al. (2001) Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. J Biol Chem 276, 31332-31339, PubMed: 11402040
49 Vabulas, R.M. et al. (2002) The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. J Biol Chem 277, 20847-20853, PubMed: 11912201
50 Zhu, F.G. and Pisetsky, D.S. (2001) Role of the heat shock protein 90 in immune response stimulation by bacterial DNA and synthetic oligonucleotides. Infect Immun 69, 5546-5552, PubMed: 11500428
51 Bandholtz, L. et al. (2003) Hsp90 binds CpG oligonucleotides directly: implications for hsp90 as a missing link in CpG signaling and recognition. Cell Mol Life Sci 60, 422-429, PubMed: 12678505
52 Triantafilou, M. et al. (2002) Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation. J Cell Sci 115, 2603-2611, PubMed: 12045230
53 Broquet, A.H. et al. (2003) Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. J Biol Chem 278, 21601-21606, PubMed: 12682040
54 Asea, A. et al. (2000) HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6, 435-442, PubMed: 10742151
55 Gao, B. and Tsan, M.F. (2003) Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70) preparation is responsible for the induction of tumor necrosis factor alpha release by murine macrophages. J Biol Chem 278, 174-179, PubMed: 12403778
56 Moriuchi, M. et al. (1998) Exposure to bacterial products renders macrophages highly susceptible to T-tropic HIV-1. J Clin Invest 102, 1540-1550, PubMed: 9788967
57 Deng, H. et al. (1996) Identification of a major co-receptor for primary isolates of HIV-1. Nature 381, 661-666, PubMed: 8649511
58 Feng, Y. et al. (1996) HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272, 872-877, PubMed: 8629022
59 Juffermans, N.P. et al. (2000) Up-regulation of

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

HIV coreceptors CXCR4 and CCR5 on CD4(+) T cells during human endotoxemia and after stimulation with (myco)bacterial antigens: the role of cytokines. Blood 96, 2649-2654, PubMed: 11023494
60 Inohara, N. et al. (2001) Human Nod1 confers responsiveness to bacterial lipopolysaccharides. J Biol Chem 276, 2551-2554, PubMed: 11058605
61 Chamaillard, M. et al. (2003) An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol 4, 702-707, PubMed: 12796777
62 Girardin, S.E. et al. (2003) Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science 300, 1584-1587, PubMed: 12791997
63 Girardin, S.E. et al. (2003) Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. J Biol Chem 278, 41702-41708, PubMed: 12871942
64 Girardin, S.E. et al. (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278, 8869-8872, PubMed: 12527755
65 Gutierrez, O. et al. (2002) Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kappa B activation. J Biol Chem 277, 41701-41705, PubMed: 12194982
66 Hugot, J.P. et al. (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411, 599-603, PubMed: 11385576
67 Ogura, Y. et al. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411, 603-606, PubMed: 11385577
68 Perera, P.Y. et al. (2001) CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and taxol-inducible gene expression. J Immunol 166, 574-581, PubMed: 11123339
69 Ozinsky, A. et al. (2000) The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci U S A 97, 13766-13771, PubMed: 11095740
70 Hajjar, A.M. et al. (2001) Cutting edge: functional interactions between toll-like receptor (TLR) 2 and TLR1 or TLR6 in response to phenol-soluble modulin. J Immunol 166, 15-19, PubMed: 11123271
71 Anderson, H.A., Hiltbold, E.M. and Roche, P.A. (2000) Concentration of MHC class II molecules in lipid rafts facilitates antigen presentation. Nat Immunol 1, 156-162, PubMed: 11248809
72 Pralle, A. et al. (2000) Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. J Cell Biol 148, 997-1008, PubMed: 10704449
73 Wang, P.Y., Kitchens, R.L. and Munford, R.S. (1995) Bacterial lipopolysaccharide binds to CD14 in low-density domains of the monocyte-macrophage plasma membrane. J Inflamm 47, 126-137, PubMed: 9117440
74 Latz, E. et al. (2002) Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction. J Biol Chem 277, 47834-47843, PubMed: 12324469
75 Hotchkiss, R.S. and Karl, I.E. (2003) The pathophysiology and treatment of sepsis. N Engl J Med 348, 138-150, PubMed: 12519925
76 Wheeler, A.P. and Bernard, G.R. (1999) Treating patients with severe sepsis. N Engl J Med 340, 207-214, PubMed: 9895401
77 Bulger, E.M. and Maier, R.V. (2000) Lipid mediators in the pathophysiology of critical illness. Crit Care Med 28, N27-36, PubMed: 10807314
78 Zimmerman, G.A. et al. (2002) The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. Crit Care Med 30, S294-301, PubMed: 12004251
79 Carvalho, A.C. and Freeman, N.J. (1994) How coagulation defects alter outcome in sepsis: survival may depend on reversing procoagulant conditions. J Crit Illness 9, 51-75
80 Levi, M. and Ten Cate, H. (1999) Disseminated intravascular coagulation. N Engl J Med 341, 586-592, PubMed: 10451465
81 Ziegler, E.J. et al. (1991) Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. N Engl J Med 324, 429-436, PubMed: 1988827
82 Alejandria, M.M. et al. (2002) Intravenous immunoglobulin for treating sepsis and septic shock. Cochrane Database Syst Rev CD001090, PubMed: 11869591
83 Goeddel, D.V. (1999) Signal transduction by tumor necrosis factor: the Parker B. Francis Lectureship. Chest 116, 69S-73S, PubMed: 10424599
84 Abraham, E. et al. (2001) Lenercept (p55 tumor

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

necrosis factor receptor fusion protein) in severe sepsis and early septic shock: a randomized, double-blind, placebo-controlled, multicenter phase III trial with 1,342 patients. Crit Care Med 29, 503-510, PubMed: 11373411
85 Fisher, C.J., Jr. et al. (1993) Influence of an anti-tumor necrosis factor monoclonal antibody on cytokine levels in patients with sepsis. The CB0006 Sepsis Syndrome Study Group. Crit Care Med 21, 318-327, PubMed: 8440099
86 Abraham, E. et al. (1995) Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome. A randomized, controlled, double-blind, multicenter clinical trial. TNF-alpha MAb Sepsis Study Group. Jama 273, 934-941, PubMed: 7884952
87 Cohen, J. and Carlet, J. (1996) INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group. Crit Care Med 24, 1431-1440, PubMed: 8797612
88 Abraham, E. et al. (1998) Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group. Lancet 351, 929-933, PubMed: 9734938
89 Clark, M.A. et al. (1998) Effect of a chimeric antibody to tumor necrosis factor-alpha on cytokine and physiologic responses in patients with severe sepsis—a randomized, clinical trial. Crit Care Med 26, 1650-1659, PubMed: 9781721
90 Penacek, E.A. et al. (2000) Neutralisation of TNF by a monoclonal antibody improves survival and reduces organ dysfunction in human sepsis: Results of the MONARCS trial. Chest 116, S88
91 Gallagher, J. et al. (2001) A multicenter, open-label, prospective, randomized, dose-ranging pharmacokinetic study of the anti-TNF-alpha antibody afelimomab in patients with sepsis syndrome. Intensive Care Med 27, 1169-1178, PubMed: 11534565
92 Fisher, C.J., Jr. et al. (1994) Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. Jama 271, 1836-1843, PubMed: 8196140
93 Opal, S.M. et al. (1997) Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med 25, 1115-1124, PubMed: 9233735
94 Levin, M. et al. (2000) Recombinant bactericidal/permeability-increasing protein (rBPI21) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial. rBPI21 Meningococcal Sepsis Study Group. Lancet 356, 961-967, PubMed: 11041396
95 Larrick, J.W. et al. (1995) Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. Infect Immun 63, 1291-1297, PubMed: 7890387
96 Flegel, W.A. et al. (1989) Inhibition of endotoxin-induced activation of human monocytes by human lipoproteins. Infect Immun 57, 2237-2245, PubMed: 2731990
97 Hanasawa, K. (2002) Extracorporeal treatment for septic patients: new adsorption technologies and their clinical application. Ther Apher 6, 290-295, PubMed: 12164799
98 Uhl, W. et al. (1995) A multicenter study of phospholipase A2 in patients in intensive care units. J Am Coll Surg 180, 323-331, PubMed: 7874343
99 Springer, D.M. (2001) An update on inhibitors of human 14 kDa Type II s-PLA2 in development. Curr Pharm Des 7, 181-198, PubMed: 11311112
100 Dhainaut, J.F. et al. (1994) Platelet-activating factor receptor antagonist BN 52021 in the treatment of severe sepsis: a randomized, double-blind, placebo-controlled, multicenter clinical trial. BN 52021 Sepsis Study Group. Crit Care Med 22, 1720-1728, PubMed: 7956274
101 Johnson, C.D. et al. (2001) Double blind, randomised, placebo controlled study of a platelet activating factor antagonist, lexipafant, in the treatment and prevention of organ failure in predicted severe acute pancreatitis. Gut 48, 62-69, PubMed: 11115824
102 Poeze, M. et al. (2000) Decreased organ failure in patients with severe SIRS and septic shock treated with the platelet-activating factor antagonist TCV-309: a prospective, multicenter, double-blind, randomized phase II trial. TCV-309 Septic Shock Study Group. Shock 14, 421-428, PubMed: 11049104
103 Schuster, D.P. et al. (2003) Recombinant platelet-activating factor acetylhydrolase to prevent acute respiratory distress syndrome and mortality in severe sepsis: Phase IIb, multicenter, randomized, placebo-controlled, clinical trial. Crit Care Med 31, 1612-1619, PubMed: 12794395

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

104 Grover, R. et al. (1999) An open-label dose escalation study of the nitric oxide synthase inhibitor, N(G)-methyl-L-arginine hydrochloride (546C88), in patients with septic shock. Glaxo Wellcome International Septic Shock Study Group. Crit Care Med 27, 913-922, PubMed: 10362413
105 Root, R.K. et al. (2003) Multicenter, double-blind, placebo-controlled study of the use of filgrastim in patients hospitalized with pneumonia and severe sepsis. Crit Care Med 31, 367-373, PubMed: 12576938
106 Gregory, S.A., Morrissey, J.H. and Edgington, T.S. (1989) Regulation of tissue factor gene expression in the monocyte procoagulant response to endotoxin. Mol Cell Biol 9, 2752-2755, PubMed: 2503712
107 Smith, O.P. et al. (1997) Use of protein-C concentrate, heparin, and haemodiafiltration in meningococcus-induced purpura fulminans. Lancet 350, 1590-1593, PubMed: 9393338
108 Bernard, G.R. et al. (2001) Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 344, 699-709, PubMed: 11236773
109 Warren, B.L. et al. (2001) Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial. Jama 286, 1869-1878, PubMed: 11597289
110 Abraham, E. (2000) Tissue factor inhibition and clinical trial results of tissue factor pathway inhibitor in sepsis. Crit Care Med 28, S31-33, PubMed: 11007194
111 Jilma, B. et al. (2002) Pharmacodynamics of active site-inhibited factor VIIa in endotoxin-induced coagulation in humans. Clin Pharmacol Ther 72, 403-410, PubMed: 12386642
112 Schumer, W. (1976) Steroids in the treatment of clinical septic shock. Ann Surg 184, 333-341, PubMed: 786190
113 Bone, R.C. et al. (1987) A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. N Engl J Med 317, 653-658, PubMed: 3306374
114 Cronin, L. et al. (1995) Corticosteroid treatment for sepsis: a critical appraisal and meta-analysis of the literature. Crit Care Med 23, 1430-1439, PubMed: 7634816
115 Annane, D. et al. (2002) Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. Jama 288, 862-871, PubMed: 12186604
116 Loppnow, H. et al. (1990) Cytokine induction by lipopolysaccharide (LPS) corresponds to lethal toxicity and is inhibited by nontoxic Rhodobacter capsulatus LPS. Infect Immun 58, 3743-3750, PubMed: 2228245
117 Rose, J.R. et al. (1995) Agonistic and antagonistic activities of bacterially derived Rhodobacter sphaeroides lipid A: comparison with activities of synthetic material of the proposed structure and analogs. Infect Immun 63, 833-839, PubMed: 7868254
118 Rietschel, E.T. et al. (1996) Bacterial endotoxin: Chemical constitution, biological recognition, host response, and immunological detoxification. Curr Top Microbiol Immunol 216, 39-81, PubMed: 8791735
119 Loppnow, H. et al. (1989) IL-1 induction-capacity of defined lipopolysaccharide partial structures. J Immunol 142, 3229-3238, PubMed: 2651523
120 Golenbock, D.T. et al. (1991) Lipid A-like molecules that antagonize the effects of endotoxins on human monocytes. J Biol Chem 266, 19490-19498, PubMed: 1918061
121 Christ, W.J. et al. (1995) E5531, a pure endotoxin antagonist of high potency. Science 268, 80-83, PubMed: 7701344
122 Kawata, T. et al. (1999) E5531, a synthetic non-toxic lipid A derivative blocks the immunobiological activities of lipopolysaccharide. Br J Pharmacol 127, 853-862, PubMed: 10433491
123 Marshall, J.C. (2003) Such stuff as dreams are made on: mediator-directed therapy in sepsis. Nat Rev Drug Discov 2, 391-405, PubMed: 12750742
124 Mancuso, G. et al. (2002) Mitogen-activated protein kinases and NF-kappa B are involved in TNF-alpha responses to group B streptococci. J Immunol 169, 1401-1409, PubMed: 12133965
125 Beutler, B. and Rehli, M. (2002) Evolution of the TIR, tolls and TLRs: functional inferences from computational biology. Curr Top Microbiol Immunol 270, 1-21, PubMed: 12467241
126 Kawai, T. et al. (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity 11, 115-122, PubMed: 10435584
127 Horng, T., Barton, G.M. and Medzhitov, R. (2001) TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol 2, 835-841, PubMed: 11526399
128 Horng, T. et al. (2002) The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. Nature 420, 329-333, PubMed: 12447442

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004

129 Hoebe, K. et al. (2003) Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. Nature 424, 743-748, PubMed: 12872135
130 Poltorak, A. et al. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282, 2085-2088, PubMed: 9851930
131 Wright, S.D. and Jong, M.T. (1986) Adhesion-promoting receptors on human macrophages recognize Escherichia coli by binding to lipopolysaccharide. J Exp Med 164, 1876-1888, PubMed: 3537192
132 Nathan, C. and Ding, A. (2001) TREM-1: a new regulator of innate immunity in sepsis syndrome. Nat Med 7, 530-532, PubMed: 11329047

Further reading, resources and contacts

An outstanding review that catalogues all the clinical trials attempted so far for sepsis:

Marshall, J.C. (2003) Such stuff as dreams are made on: mediator-directed therapy in sepsis. Nat Rev Drug Discov 2, 391-405

The website of the International Endotoxin Society (IES):

http://www.kumc.edu/IES/

Features associated with this article

Figures

Figure 1. Models of lipopolysaccharide (LPS) recognition.
Figure 2. The coagulation cascade in sepsis.
Figure 3. Potential therapeutic targets for sepsis.

Tables

Table 1. Pattern recognition receptors (PAMPs) involved in lipopolysaccharide (LPS) recognition.
Table 2. Therapeutic interventions for septic shock.

Citation details for this article

Martha Triantafilou and Kathy Triantafilou (2004) Sepsis: molecular mechanisms underlying lipopolysaccharide recognition. Expert Rev. Mol. Med. Vol. 6, 13 February, DOI: 10.1017/S1462399404007409

Accession information: DOI: 10.1017/S1462399404007654; Vol. 6; 13 February 2004