Strategies for the control of LPS-mediated pathophysiological disorders

Richard Chaby

Lipopolysaccharides released from Gram-negative bacteria after infection initiate an alarm response in the host, which has supposedly evolved to protect it. However, an exaggerated response leads to a cascade of pathophysiological events termed sepsis. In the USA alone, the annual number of deaths caused by sepsis (~70,000) is comparable with that caused by AIDS. The author describes the major advances of knowledge in this field and the attempts to convert this into successful therapeutics. Anti-endotoxin and anti-inflammatory agents have been disappointing, but new strategies might result in effective treatments in the forthcoming years.

When a host recognizes a component of a potential pathogen as 'non-self', an efficient alert system triggers the innate immune response. This rather primitive and nonspecific mechanism[1] is able to destroy invading pathogens, but it can also, if over stimulated, initiate toxic effects against the host. The toxic effects of Gram-negative bacteria are due to a non-secreted, heat-stable endotoxin, also termed lipopolysaccharide (LPS) because it consists of carbohydrates and lipids. LPS is a unique glycolipid found exclusively in the outer leaflet of the outer membrane of Gram-negative bacteria. The structure of LPS was elucidated in the 1980s by different groups[2,3]; it consists of three regions: the lipid region (termed lipid A); the core oligosaccharide, which is linked to the lipid A via the acidic deoxysugar 3-deoxy-D-*manno*-octulosonic acid (Kdo); and a repeating oligosaccharide polymer (termed O antigen) that extends from the polysaccharide core (Fig. 1). Lipid A consists of a glucosaminyl-β1′-6-glucosamine disaccharide, which is phosphorylated at positions 1 and 4′, and acylated with hydroxylated and non-hydroxylated fatty acids. One Gram-negative bacterium carries ~2×10^6 LPS/lipid A molecules, which contain 25% of the fatty acyl chains of the envelope. The minimal LPS structure required for the growth of Gram-negative bacteria (termed Re-LPS) consists of lipid A and two Kdo moieties. LPS derived from wild-type isolates (smooth-type LPS) also contain additional sugars (6–8) that constitute the nonrepeating core region, and an O-antigen side chain consisting of 1 to 50 repeats of an oligosaccharide (1–5 sugars) (Fig. 1).

The introduction of bacterial components to the circulation is caused by infections, frequently nosocomial, that occur in immunocompromised individuals, such as the elderly, patients under immunosuppressive therapy and those suffering from severe burn or trauma. The bacterial components released in the bloodstream can then lead to a fatal syndrome known as septic shock. There are ~500,000 new episodes of septic shock each year in the USA, with an associated mortality of 35% (death rate of 7.9 per 100,000 population). This mortality has escalated over the past four decades and now represents the 13th major cause of death in the USA (80,000–100,000 deaths per year). Approximately 50–60% of septic-shock episodes are associated with Gram-negative bacteremia, and particularly with their LPS component[4]. The dramatic effect of LPS in humans has been definitely demonstrated by the

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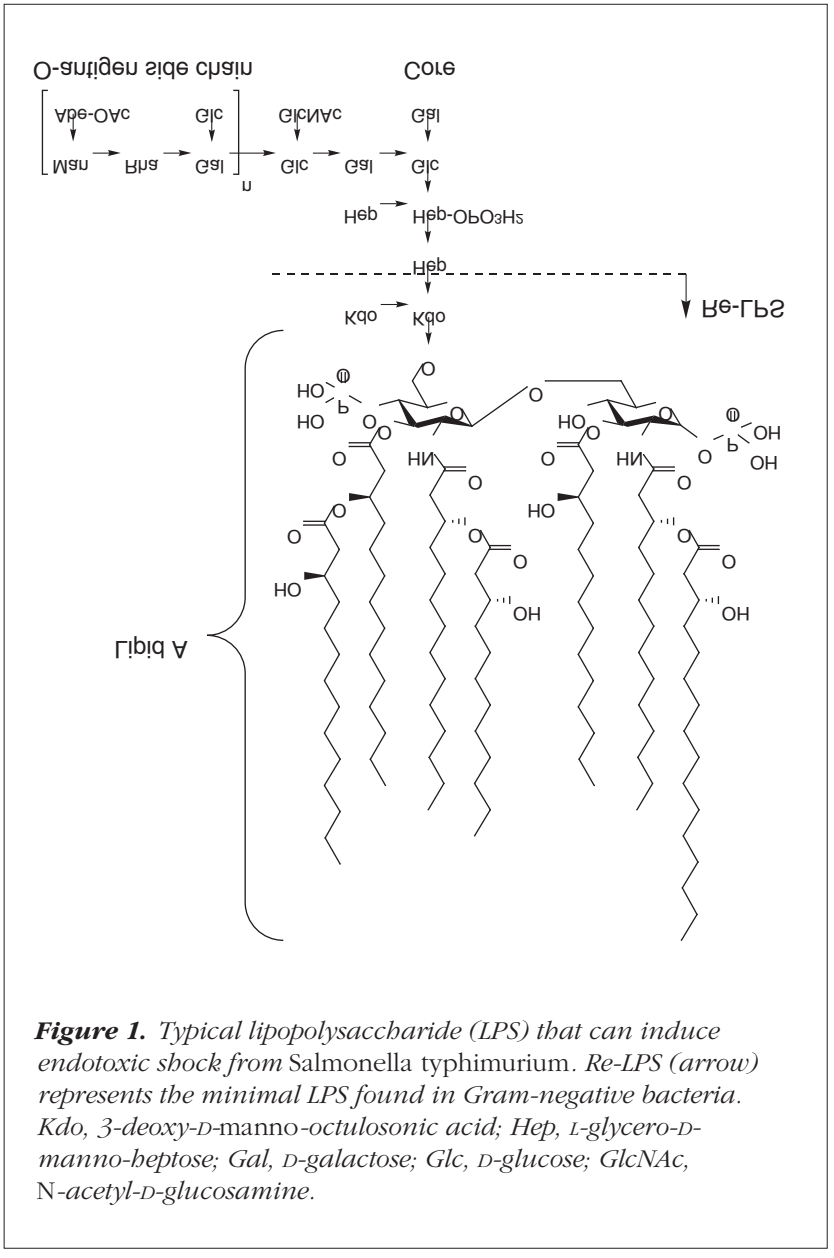

Figure 1. *Typical lipopolysaccharide (LPS) that can induce endotoxic shock from* Salmonella typhimurium. *Re-LPS (arrow) represents the minimal LPS found in Gram-negative bacteria. Kdo, 3-deoxy-D-*manno*-octulosonic acid; Hep, L-glycero-D-manno-heptose; Gal, D-galactose; Glc, D-glucose; GlcNAc,* N*-acetyl-D-glucosamine.*

severe shock that affected a laboratory worker, three hours after intravenous auto-administration of 1 mg purified LPS, in a suicide attempt. The dose was 3750 times higher than that usually used with human volunteers (4 ng kg^{-1}). The patient survived but needed treatment with fluid and epinephrine for 50 hours[5]. All of the pathological effects seen with LPS can be induced by its lipid region alone, as demonstrated with chemically synthesized lipid A (Ref. 3).

Sequence of molecular, cellular and physiological events

Although many gaps still remain in our knowledge of the responses to LPS, some of the events involved in this cascade have been clearly identified. In the bloodstream, LPS exerts humoral and cellular effects. At the humoral level, LPS activates the complement cascade and modifies different pathways of the coagulation system. It enhances fibrin formation (via activation of tissue factor and factor X, and consumption of antithrombin III) and reduces fibrin removal (by increasing the plasma levels of plasminogen activator inhibitor type 1). The clinical picture resulting from these derangements of the coagulation system, with simultaneous occurrence of thrombosis and bleeding is called disseminated intravascular coagulation. In addition to its humoral effects, LPS stimulates several cell types to secrete active molecules. After interaction with LPS: B cells are polyclonally activated and secrete immunoglobulins; mast cells and basophils produce chemotactic factors, histamine and serotonin; platelets secrete growth and coagulation factors; and neutrophilic granulocytes release active oxygen species. Inflammatory cytokines [tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and IL-6] – the major class of multipotent mediators of LPS effects – are essentially produced by monocytes, macrophages and endothelial cells.

The central role of monocytes and macrophages raises two basic questions: how are picogram quantities of LPS recognized by these cells, and how are the signals necessary for the production of cytokines transduced? It has been recognized for some time that sensitive responses of monocytes and macrophages to LPS require at least two biochemical elements: an LPS-binding protein (LBP), which is an acute-phase reactant, and a cell receptor known as CD14 (Ref. 6). Because of the amphipathic nature of LPS and its tendency to form micelles in an aqueous environment, circulating endotoxin is often an aggregate of LPS molecules. LBP, the high-affinity LPS-binding plasma component, dissociates LPS aggregates for presentation of the resulting LPS–LBP complex to CD14 (Ref. 7; Fig. 2). However, CD14 is a glycoprotein tethered to the membrane of myeloid cells by a glycosyl-phosphatidylinositol (GPI) anchor. Because the GPI tail does not traverse the cell membrane for direct connection to the signal-transduction apparatus, another cell membrane component acting as a co-receptor must be involved. Recently, Janeway and colleagues[8] found a homolog of a

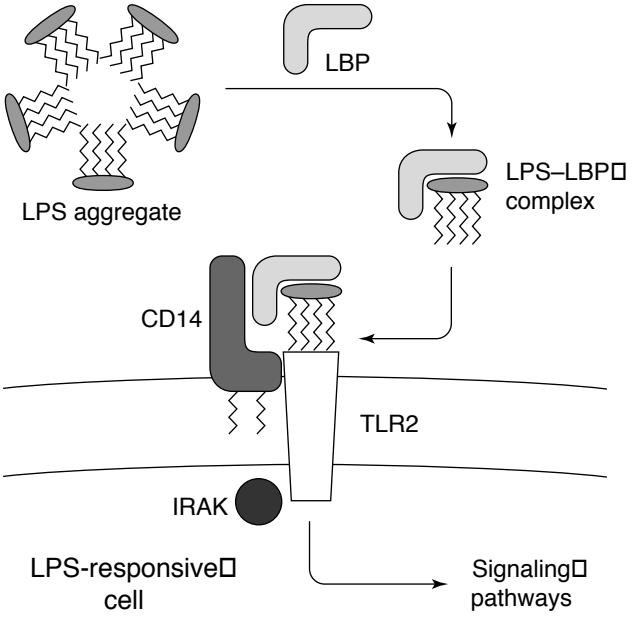

Figure 2. *Activation of monocytes and macrophages by lipopolysaccharide (LPS). LBP, LPS-binding protein; TLR2, toll-like receptor 2; IRAK, interleukin 1-receptor accessory protein kinase.*

Drosophila receptor called Toll on cells of the human immune system. Five human Toll-like receptors (TLR1 to TLR5) have been identified, and one of these (TLR4) can activate nuclear factor κ-binding (NF-κB) and induce the expression of cytokines. Subsequently, Yang and colleagues[9] demonstrated that another protein of this family, TLR2, which is expressed in all lymphoid tissues, binds LPS by its extracellular domain, and transduces LPS responsiveness by a region of its intracellular domain required for association with the IL-1-receptor accessory protein kinase. Therefore, TLR2 appears to be the co-receptor of CD14 and the direct mediator of signaling by LPS and induction of cytokine production (Fig. 2).

Another important participant in the septic shock cascade is neutral endopeptidase (NEP), a metalloproteinase expressed by inflammatory cells that terminates the action of various proinflammatory cytokines. NEP-deficient mice show an increased sensitivity to TNF-α and IL-1, and an enhanced lethality to endotoxic shock[10].

Once released from LPS-stimulated cells, cytokines can activate cells of different tissues and organs (leukocytes, brain, adrenals and liver), inducing metabolic, hormonal and neuroendocrine alterations. Cytokines, hormones and neurotransmitters are, thus, the primary mediators of the

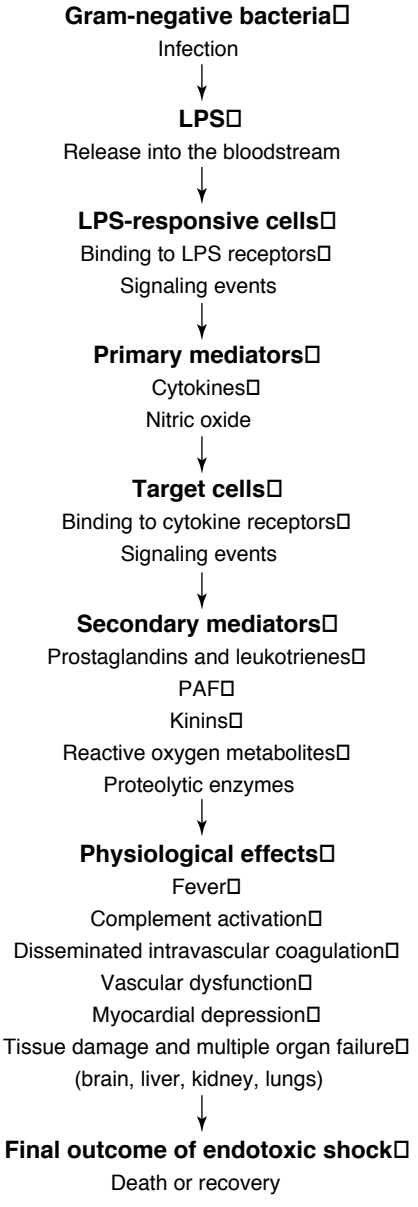

Figure 3. *Simplified scheme of the endotoxic shock cascade.*

cascade of events that occur after endotoxemia. Secondary mediators, such as prostaglandins and leukotrienes released from neutrophils, and nitric oxide produced in large amounts by monocytes and macrophages, also play a key role because of their potent local action on the microvasculature. Many vascular beds are dilated whereas others are constricted.

The conjunction of vascular abnormalities and intravascular coagulation result in the abnormal distribution of

blood flow, inadequate perfusion and reduced oxygen delivery to various tissues. This might contribute to cell dysfunction and the progressive failure of different organs, termed multi-organ failure or multi-organ dysfunction syndrome. The organ systems affected are the brain (sequential alterations of mental status, with agitation, stupor and coma), heart (myocardial depression), kidneys (acute renal failure), liver (hepatic failure) and lungs (acute respiratory distress syndrome, ARDS).

A simplified linear model of the cascade of events associated with Gram-negative bacteremia is represented in Fig. 3. Although the real cascade is certainly much more complex, with intricate loops and great redundancy, the linear model in Fig. 3 can help to identify the potential targets for therapeutic intervention, and to understand the principles supporting the various strategies[11] that have been investigated.

Inhibition of bacterial LPS biosynthesis or LPS release

Because endotoxemia is the consequence of the proliferation of Gram-negative bacteria at a site of infection, early antibacterial therapy is required. A late treatment with conventional antibiotics is not useful because it accelerates the release of LPS, which, in turn, aggravates the disease[12]. However, the observation that mutations in LPS biosynthesis render Gram-negative bacteria more susceptible to clearance by the host suggested to some investigators that inhibitors of LPS biosynthesis could be more effective than other antibiotics against endotoxic shock. An inhibitor that interferes with the synthesis of the core region of LPS has been designed[13]. It is an α-C-(1,5-anhydro-7-amino-2,7-dideoxy-D-*manno*-heptopyranosyl)-carboxylate attached to the carboxyl terminus of a dipeptide by an amide linkage (Fig. 4). This compound blocks LPS synthesis by inhibiting CMP-Kdo synthetase. Thus, bacterial growth is inhibited and they become strikingly more susceptible to complement killing. Another agent, L161240, that interferes with the synthesis of the lipid A region of LPS, has been developed. This compound inhibits the UDP-3-*O*-acyl-*N*-acetylglucosamine deacetylase, is bactericidal against various Gram-negative bacteria, and protects mice from a lethal dose of *Escherichia coli*[14]. However, clinical trials with these two inhibitors of LPS biosynthesis, or investigations on their influence on the release of LPS, have not been reported.

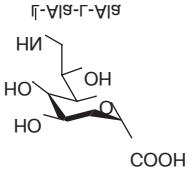

Figure 4. *Inhibitor of the biosynthesis of 3-deoxy-D-manno-octulosonic acid, a component that links the polysaccharide and lipid A regions of lipopolysaccharide.*

Detoxification or removal of endotoxin

Once in the bloodstream, the natural route of clearance of endotoxin is its degradation in the liver, particularly by Kupffer cells[15]. This natural mechanism can be enhanced by administration of triglyceride-rich lipoproteins[16] or carboxymethyl-1-β-1,3-glucan, a ligand of the scavenger receptor[17].

Direct removal of LPS from the circulation can also be attempted. Although endotoxins have relatively low molecular size, they are inefficiently removed by hemodialysis, but the use of blood or plasma exchange procedures (plasmapheresis) improves the survival rate[18]. More specific procedures to remove LPS, such as extracorporeal filtration over polymyxin B immobilized fibers, also reduce organic disorders[19]. This direct hemoperfusion technique is currently in clinical use in Japan.

Neutralization of circulating LPS

Anti-LPS antibodies

Early therapy targeting the neutralization of LPS before its interaction with responsive cells is probably the best approach for preventing endotoxic shock. By analogy with the neutralization of other non-self components, considerable efforts have been focused on antibodies. It appeared that antibodies directed to the outer polysaccharide region (O antigen) of LPS are very efficient against shock, but are specific to a bacterial strain. Therefore, because of the difficulty in early identification of the infectious agent, the utility of a therapy with such specific antibodies is considerably reduced. However, because only a few common Gram-negative bacteria cause the majority of human infections, the prophylactic administration of a polyvalent antiserum, consisting of a mixture of specific antibodies, is sometimes useful in selected patients[20].

To provide a broad cross-protection against LPS from various bacteria, another strategy is to use antibodies directed towards the more conserved core and/or lipid A regions of LPS. It is noteworthy that all sera contain such antibodies. It has been shown that a high level of pre-existing anti-core/lipid A IgM is associated with reduced mortality

from sepsis among surgical patients[21]. The level of these antibodies can be enhanced by vaccination with detoxified LPS preparations from rough mutants of Gram-negative bacteria[22], or by prophylactic administration of polyclonal antibodies. However, it has been shown that the protection of septic patients treated with a human anti-*E.coli* J5 antiserum did not correlate with hemagglutinating antibody titers[23].

As the efficiency of polyclonal anti-LPS antibodies is low, and their exact role in the protection is not clear, another strategy has been to develop monoclonal antibodies (mAbs) against the core or lipid A regions. One of the first mAbs directed against lipid A has been developed by Girard and Chaby[24]. After this, anti-Kdo (Ref. 25) and several other anti-core/lipid A mAbs were described, including HA-1A, E5, T88, SdJ5 1.17.15, D6B3, 8-2/26-20, GL11 and MLA-1 (Ref. 26). Among these, only HA-1A (human IgM from Centocor, Leiden, The Netherlands), E5 (murine IgM from Xoma, Berkeley, CA, USA) and T88 (human IgM from Chiron, Emeryville, CA, USA) have been evaluated in large-scale clinical trials. The clinical studies documented a lack of benefit with these mAbs, and none of them can now be recommended for the treatment of Gram-negative sepsis. A chimeric IgG1 mAb (SDZ219800) has also been developed by Sandoz (Basel, Switzerland), but awaits investigations in large clinical trials.

One possible explanation for these disappointing results could be that the biologically active lipid A region of LPS is often inaccessible to the antibodies. On intact Gram-negative bacteria, lipid A is inserted into the outer membrane. In the bloodstream, LPS is found either as LPS aggregates, or associated to circulating lipoproteins or acute-phase reactants. Therefore, in all these situations, lipid A is in a cryptic position and cannot be easily reached by antibodies. Furthermore, even when the mAb actually binds to lipid A, neutralization of the endotoxic action does not necessarily follow. For example, neutralization could not be proven for the anti-lipid A mAb E5 (Ref. 27).

Agents from bacteria, insects, crabs and amphibians

Instead of antibodies, exogenous or endogenous LPS-binding proteins and peptides can also be used as potential LPS-neutralizing agents. LPS-binding proteins produced by bacteria, or present in the hemolymph of insects and crabs, and in amphibians, are potential candidates for neutralization of LPS. Polymyxin B is a cyclic polycationic decapeptide from *Bacillus polymyxa* (a Gram-positive bacterium), which interacts via its positively charged primary amines (hydrophilic interactions) and its fatty acid (hydrophobic interactions) with the negatively charged Kdo-lipid A region of LPS. However, toxic effects limit its clinical use. In insect hemolymph, lipophorin (a lipoprotein), and cecropin peptides, can bind LPS, probably by its lipid A region[28]. Anti-LPS factors from the amebocytes of the horseshoe crab *Limulus polyphemus* also bind avidly to LPS (Ref. 29). One of these, an 11.8 kDa molecule termed endotoxin-neutralizing protein (ENP), can protect rabbits from Gram-negative sepsis[30]. In amphibians, magainins isolated from *Xenopus* skin are other polycationic peptides that can interact with LPS and reduce some of its effects[31]. The drawback with all these exogenous proteins is that they are highly immunogenic. This limits their potential therapeutic utility.

Host components from serum or leukocytes

There are several host-derived molecules described that can bind LPS. Whereas some of these endogenous molecules (albumin, transferrin, lactoferrin, hemoglobin, lysozyme and mannose-binding protein) do not modify LPS bioactivity, others do. These include: serum components such as lipoproteins (LDL, HDL) and serum amyloid P (SAP); acute-phase proteins produced by the liver, such as the LPS-binding protein (LBP)[32]; and granulocyte proteins such as indolicidins[33], defensins[34], an 18 kDa cationic antimicrobial protein (CAP18)[35], azurocidin (CAP37)[36] and the bactericidal permeability-increasing protein (BPI, also termed CAP57)[37].

Various lipoproteins, including chylomicrons, VLDL, LDL and HDL, bind and inactivate LPS. Administration of HDL to human volunteers blocked LPS-induced inflammation efficiently[38], probably via hepatic clearance of HDL–LPS complexes. The soluble form of CD14, which shuttles LPS to HDL (Ref. 39), can also be used to enhance LPS clearance, but might also enhance LPS-induced production of inflammatory cytokines by endothelial cells. Serum Amyloid P (SAP), and a 13-amino-acid peptide derived from it ($SAP_{27–39}$) can also neutralize LPS (Ref. 40). The granulocyte-derived protein CAP18 can bind LPS and inhibit several of its activities. It has been shown recently[41] that a synthetic 27-amino-acid peptide ($CAP18_{109–135}$) from the C-terminal LPS-binding domain of CAP18, protects mice from LPS-induced death. But the most promising host-derived factor for the treatment of endotoxic shock is probably BPI. This 55–60 kDa azurophilic granule protein has a 45% sequence homology with LBP (Ref. 42). However, LBP enhances LPS activities whereas BPI blocks these activities. A chimeric molecule devised to exhibit a longer circulating half-live, and consisting of an LBP–BPI fusion protein, retained the LPS-neutralizing capacity in an animal model of endotoxic shock. Recently, an evaluation of the 21 kDa N-terminal portion of recombinant human

BPI (rBPI-21) suggested overall benefit in children with meningococcal sepsis[43] – it is now under controlled Phase III clinical trial.

Prevention of the presentation and binding of LPS to its receptors

Anti-LBP and anti-CD14 antibodies

By contrast with LBP, which disperses LPS aggregates, BPI enhances the apparent size of LPS aggregates. Moreover, BPI has a stronger affinity for LPS than does LBP and, thus, inhibits LPS–LBP binding and its presentation to membrane CD14 (Ref. 44). Therefore, BPI can reduce endotoxic shock either by direct neutralization of LPS, as mentioned above, or by inhibition of the presentation of LPS to cells by appropriate shuttles, such as LBP or soluble CD14.

A second strategy to prevent LBP/CD14-mediated binding of LPS to cells is to use anti-LBP or anti-CD14 antibodies. Anti-LBP antibodies protect against endotoxic shock either by preventing the binding of LPS to LBP, or by mediating the binding of LPS–LBP complexes to complement or Fc receptors on phagocytic cells. Direct inhibition of LPS binding with anti-CD14 antibodies is also possible. This potential clinical treatment is under evaluation. However, it should be pointed out that soluble forms of CD14 shuttle LPS to HDL and thus participate in LPS clearance. One possible drawback of the use of anti-CD14 antibodies would be to inhibit this clearance pathway.

Natural and synthetic LPS antagonists

Because activation of some LPS-responsive cells is not, or not exclusively, mediated by CD14, a less restrictive strategy to prevent LPS binding consists of the occupancy of LPS receptors with inactive competitors (antagonists) of LPS. To obtain molecules that can interact with lipid A receptors while being devoid of agonistic activity, variations in the lipid A structure are required. Such compounds (Fig. 5) can be:

- Found among the biosynthetic precursors of lipid A (lipid X, lipid IV_A)
- Produced by chemical or enzymic modification of a mature, active form of lipid A (monophosphoryl lipid A, deacylated lipid A)
- Found in some natural LPS molecules with atypical lipid A structures (*Rhodobacter capsulatus*, *Rhodobacter sphaeroides*)
- Chemically synthesized (synthetic lipid A analogs)

The two precursors of lipid A biosynthesis, lipid X and lipid IV_A (Refs 45,46) were the first reported competitive antagonists of LPS. Another incomplete lipid A derivative, monophosphoryl lipid A, confers marked protection when administered before live *E. coli* [47]. Complete LPSs from *R. capsulatus* and *R. sphaeroides*, and their diphosphoryl lipid A fragments, are also potent LPS antagonists[48].

The difficulty with all the naturally derived antagonists listed above is to obtain sufficient amounts of material with pharmaceutically acceptable purity, reproducibility and stability. Another way to obtain these molecules is to use organic synthesis. Several monosaccharide or disaccharide analogs of lipid A have been synthesized by our group[49,50] and in other laboratories[51–53]. Among diglucosamine-based compounds, PE4 (Ref. 46) and DT5461 (Ref. 52) are analogs of *E. coli* lipid A, whereas E5531 (Ref. 53) is based on the structure of *R. capsulatus*. The complete absence of agonistic activity of the latter compound is because of the presence of stable ether-linked chains at the C3 and C3′ positions of the molecule (Fig. 5). These three compounds reduce endotoxin shock in different animal models. E5531, which also blocks systemic and cardiovascular effects of LPS in human volunteers, seems particularly promising and is presently under clinical evaluation. Among monosaccharide-based lipids, a Phase I trial of SDZMRL953 indicated that pretreatment with this compound downregulates the human cytokine response to LPS (Ref. 54). Another synthetic glycophospholipid, compound SDZ880431 (also termed PPDm2) is based on the atypical structure of the lipid A of *Rhodopseudomonas viridis*, which contains 2,3-diaminoglucose instead of glucosamine. PPDm2 was shown to be a good inhibitor of LPS effects *in vitro*[51]. A derivative of PPDm2, termed PPDm2B and bearing a 6-aminocaproyl side chain as spacer arm, has been synthesized[50]. Unpublished observations suggest that PPDm2B could be a promising tool for studies of LPS receptors present on B cells and granulocytes.

Inhibition of plasma cascades activated by LPS

Kinins

Interaction of lipid A with a Hageman factor–prekallikrein complex initiates the generation of bradykinin, a nonapeptide that induces vascular pathophysiological events such as enhancement of vascular permeability. The bradykinin antagonist CP0127 showed protective activity in septic animals[55]. Two receptors, B1 and B2, are involved in bradykinin-induced effects. It has been shown that upregulation of receptor B1, or blockade of receptor B2, attenuates LPS-induced organ failure, lung dysfunction and mortality. Therefore, induction of B1 upregulation by administration of a B1 agonist can also be of therapeutic benefit[56].

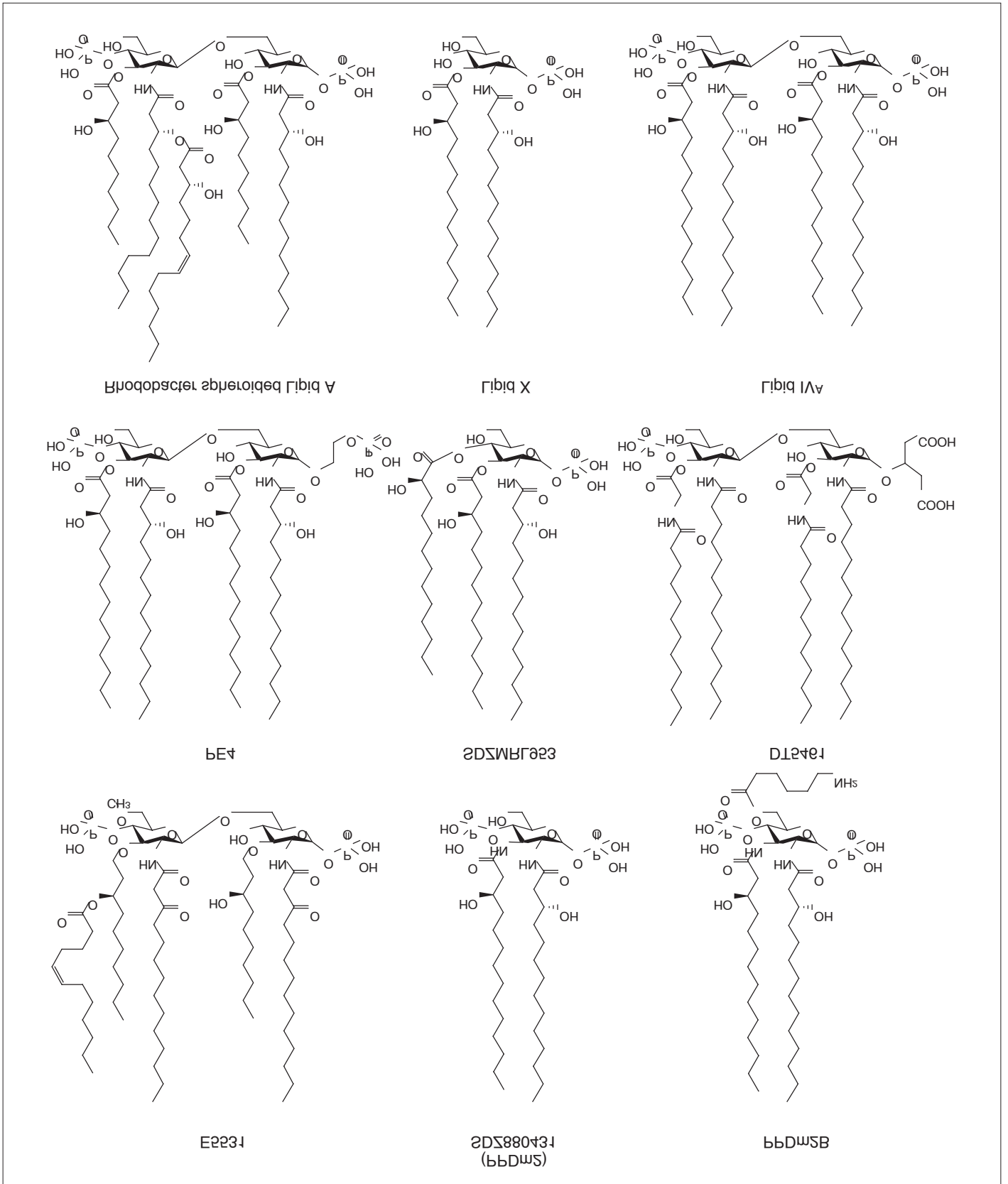

Figure 5. *Natural and synthetic antagonists of lipopolysaccharide.*

Coagulation

The coagulation system is also activated by LPS, either via the extrinsic (tissue factor) or the intrinsic (factor XII) pathways – this is why heparin reduces mortality in some animal models. As a cascade of proteases is involved in the coagulation pathways, antiproteases might also have beneficial effects. Protease inhibitors, such as hirudin, antithrombin III, eglin C and aprotinin, can partly prevent endotoxic shock in pigs[57]. Synthetic protease inhibitors, such as gabexate mesilate and nafamstat mesilate, have also been designed to reduce some of the pathophysiological effects of LPS. When administred intraperitoneally in rats, the latter (6-amidino-2-naphthyl-*p*-guanidinobenzoate dimethylsulfonate) inhibits coagulation factors VIIa and thrombin, and prevents the pulmonary vascular injury and coagulation abnormalities induced by LPS (Ref. 58).

Complement

The complement system (predominantly the alternative pathway) is also activated in septic shock patients. C1q has been reported to interact with the Kdo region of LPS, but not with lipid A (Ref. 59). Activation of C3a, C4a, C5a and C5b-9 is also observed. It has been shown that the third component C3 actually protects against endotoxic shock[60]. On the other hand, some components, such as C5a, when activated, can attract neutrophils and trigger the release of locally active agents such as leukotrienes. Therefore, neutralization of C5a by polyclonal antibodies attenuates the septic state[61].

Inhibitors of signal transduction

The problem with the use of LPS receptor antagonists is that they must be given very early, because antagonists are more efficient before the binding of LPS to effector cells. Another approach is to block LPS signal transduction. This action takes place before signal amplification and could, thus, be very potent. Several signaling pathways, implicating various protein kinases (PKC, PTK, MAP kinases and proline-directed protein kinases) have been described in signal transduction by LPS (Ref. 62). These transduction cascades generate proinflammatory cytokines (IL-1β, IL-6, TNF-α) and other mediators (nitric oxide, NO) that, in turn, trigger secondary cascades in target cells. Such secondary cascades include activation of phospholipases [PLA_2, phosphatidylcholine (PC)-PLC, PLD, phosphatidic acid (PA) hydrolase] and subsequent release of lipid mediators that can activate another set of protein kinases, leading to cytotoxicity via a signaling route implicating diacylglycerol (DAG), sphingomyelinase, ceramide and NF-κB activation[63]. Some inhibitors of these intracellular signal mechanisms can be potential therapeutic agents.

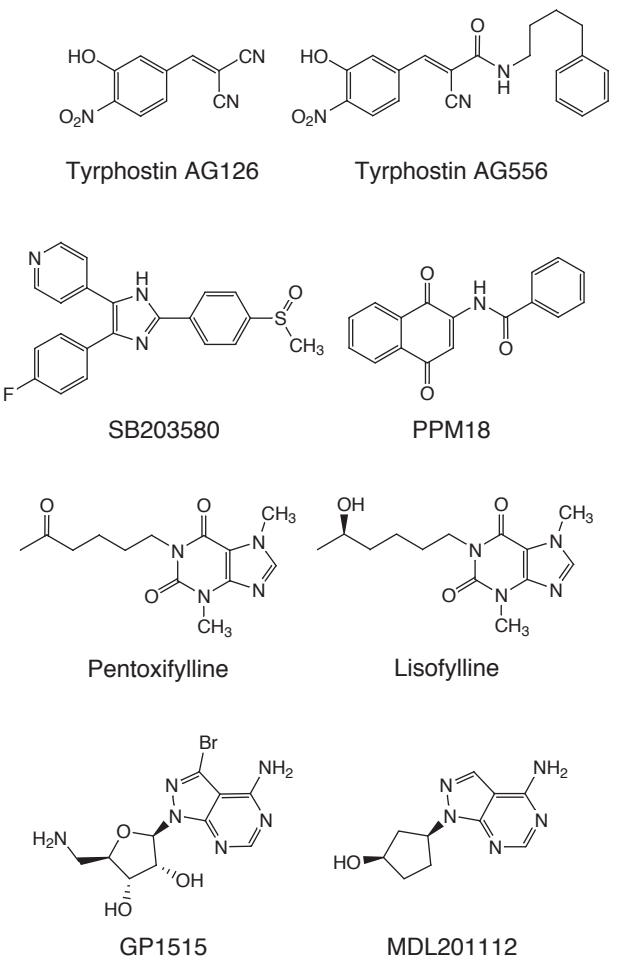

Figure 6. *Signal transduction inhibitors that have protective effects on endotoxic shock. Tyrphostins AG126 and AG556 are tyrosine kinase inhibitors. SB203580 is an inhibitor of the p38 mitogen-activated protein kinase. PPM18 is a stabilizer of the nuclear factor κ-binding inhibitor IκBα. Pentoxifylline is an inhibitor of phosphodiesterases. Lisofylline blocks excessive production of phosphatidic acid. GP1515 is an adenosine kinase inhibitor, and MDL201112 inhibits* S*-adenosyl homocysteine hydrolase.*

Inhibitors of protein kinases, NF-κB and phosphodiesterases

Because a number of kinases are involved in LPS intracellular signal transduction, several kinase inhibitors have good therapeutic value. This is particularly true for the PKC inhibitor H-7 (Ref. 64), the tyrosine kinase inhibitors genistein and tyrphostins AG126 and AG556 (Ref. 65), and the p38 MAP kinase inhibitor SB203580 (Ref. 66; Fig. 6).

NF-κB participates in the regulation of multiple cellular genes involved in the inflammatory responses. Pyrrolidine dithiocarbamate, an inhibitor of NF-κB (Ref. 67), and α-benzoylamino-1,4-naphthoquinone (PPM18), a stabilizer of the NF-κB inhibitor IκBα (Ref. 68; Fig. 6), both exert beneficial effects in sepsis models.

Inhibition of phosphodiesterases with several xanthine derivatives results in increased intracellular levels of cAMP and, by an undetermined mechanism, in decreased expression of TNF-α mRNA. Among phosphodiesterase inhibitors of this type, pentoxifylline has been reported to inhibit the release of TNF-α induced by LPS in human volunteers[69] and to increase survival in animal models of endotoxic shock. However, the high doses of pentoxifylline required for reduction of TNF-α levels lead to some toxic effects. Although intravenous preparations of pentoxifylline are available in Europe, they are not licensed in the USA, and this might limit the design of future clinical trials with this agent.

Adenine derivatives

In several studies, direct or indirect crosstalk between purinoceptor-mediated signaling and LPS signaling have been reported[70]. In this connection, compounds containing the adenine base appeared able to modulate LPS effects. It has been shown that the purine analog 2-methylthio-ATP protected mice from LPS in mortality studies[71]. Protective effects of 2-chloro-ATP and other adenosine derivatives[72] including the adenosine kinase inhibitor GP1515 (Ref. 73; Fig. 6), have also been reported. The adenyl carbocyclic nucleoside MDL201112 (a synthetic analog of adenosine and of the fungal metabolite aristeromycin; Fig. 6) has been reported to protect mice from the lethal effect of LPS (Ref. 72), presumably via inhibition of the *S*-adenosyl homocysteine hydrolase, and further inhibition of transcription of the TNF-α gene.

Inhibitors of lipid-mediated pathways

The activation of phospholipases and PA hydrolase by LPS generates lipid mediators, such as free arachidonate, lysophospholipids, PA and DAG. Inhibition of PC catabolism by tricyclodecan-9-yl-xanthogenate (compound D609), a selective PC-PLC inhibitor[74], and of PA production by lisofylline[75] (Fig. 6), can furnish protection against endotoxic shock. The free arachidonate generated by phospholipases can in turn be used in the 5-lipoxygenase pathway to generate leukotrienes (C_4, D_4 and E_4) that alter vascular permeability. A highly potent inhibitor of leukotriene biosynthesis, compound MK886, appeared to be beneficial against endotoxic shock[76].

Anti-inflammatory strategies

Therapeutics directed against cytokines

The levels of several cytokines (IL-1, IL-6, IL-8 and TNF-α) are enhanced during sepsis. The best correlation of plasma cytokine levels with mortality from septic shock has been found with IL-6. Actually, elevated levels of IL-6 are strictly determined by biologically active IL-1 and TNF-α, which cannot be directly measured because plasma contains inhibitors of IL-1 (IL-1Ra) and TNF-α (soluble TNF-α receptors p55 and p75). Many of the biological effects observed during sepsis can be mimicked by IL-1 and TNF-α, whereas IL-6 can be given to humans in very high concentrations without effects other than headache and fever, thus supporting the concept that IL-6 does not have a causal role in septic shock. However, its involvement in conjunction with other factors cannot be excluded. IL-8, which is also controlled by TNF-α, has neutrophil-activating and chemoattractant properties and, as such, is involved in neutrophil-mediated events associated with sepsis, although its exact contribution remains unclear.

Among drugs that suppress the production of cytokines, the most studied are corticosteroids. However, controlled clinical trials have now clearly demonstrated that corticosteroids do not improve survival in humans[77]. Other inhibitors of cytokine production are natural agents, such as anti-inflammatory cytokines (IL-4 and IL-10), or inhibitors of enzymes required for the processing of cytokines to their mature forms (calpain for IL-1α, IL-1β-converting enzyme for IL-1β and zinc metalloproteinases for TNF-α). Agents able to neutralize cytokines can also be used, such as the IL-1 receptor antagonist (IL-1Ra), soluble forms of the TNF-α receptors (p55 and p75) and neutralizing antibodies to IL-1 and TNF-α. Finally, because the neuroendocrine system is a powerful regulator of immune and inflammatory reactions, neurohormones of the hypothalamus–pituitary–adrenal axis are also natural modulators of the actions of cytokines. The adrenocorticotropic hormone inhibits cytokines via glucocorticoid production, and the α-melanocyte-stimulating hormone (α-MSH), which antagonizes the pyrogenic and pro-inflammatory effects of cytokines, was found to prevent endotoxic shock. A synthetic heptapeptide analog of α-MSH (HP-228) has been shown to be even more protective in animal models[78].

However, inhibitors of cytokines must be administered with caution because cytokine effects are complex and interdependent. Removal of cytokines might be more harmful than beneficial because they often represent a compensatory response of the host, with salutary effects. For example, sublethal injections of TNF-α induce a kind of endotoxin tolerance that protects animals against subsequent administration

of LPS (Ref. 79). Furthermore, removal of TNF-α and IL-1 leave the host immunocompromised. This might explain why, in clinical situations, therapeutics directed against inflammatory cytokines have been so disappointing.

NO production and NO synthase antagonists

Nitric oxide is produced in large quantities by inducible NO synthase (iNOS) in endothelial and smooth vascular muscle cells a few hours after exposure to LPS (Ref. 80). The central pathophysiological role of iNOS expression in sepsis has been demonstrated in recent studies showing that iNOS knockout mice are significantly resistant to LPS-induced septic shock[81]. NO synthase inhibitors such as aminoguanidine[82] and N^G-monomethyl-L-arginine[83] improved survival in models of endotoxin shock. The major problem in the use of NOS inhibitors is that intact endothelial NOS (eNOS) activity is necessary to counteract platelet aggregation, vasoconstriction and decreased cardiac performance. Therefore, only selective iNOS inhibitors that do not block eNOS should be used. A potent endogenous inhibitor of this type is transforming growth factor β (TGF-β) (Ref. 84). This agent does not inhibit the enzyme activity of iNOS, but reduces its induction. Therefore, treatment with TGF-β is only useful early in the induction phase. However, its systemic administration causes tissue fibrosis, which limits its clinical use. Another promising agent is 2,4-diamino-6-hydroxypyrimidine (DAHP). This agent inhibits the synthesis of tetrahydrobiopterin, which is required for assembly of active iNOS dimers. Treatments with DAHP significantly improve the survival of septic rats[85].

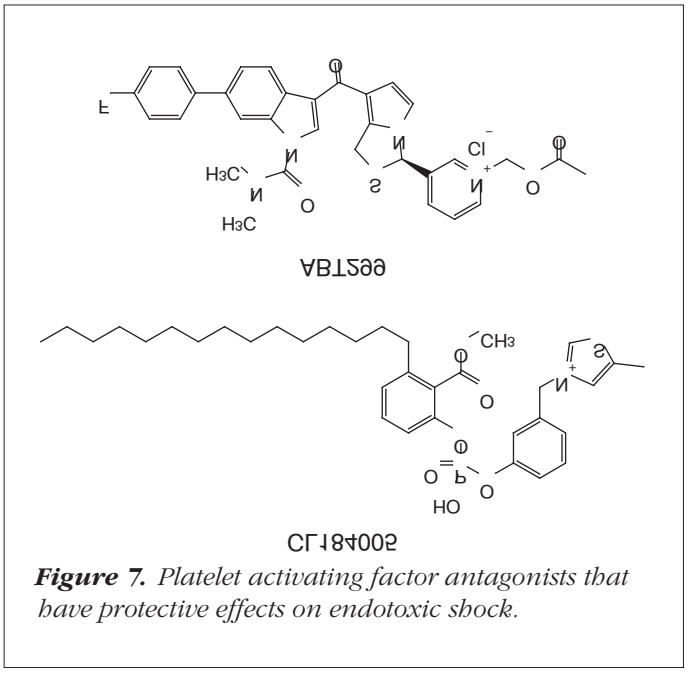

Figure 7. *Platelet activating factor antagonists that have protective effects on endotoxic shock.*

PAF antagonists

Platelet activating factor (PAF) is an endogenous phospholipid [1-*O*-alkyl-2-(*R*)-acetyl-*sn*-glyceryl-3-phosphonocholine] synthesized by a variety of inflammatory and non-inflammatory cells. Elevated levels of PAF in the blood of LPS-treated rats and septic patients, induction of a sepsis state after administration of PAF, and attenuation of sepsis with PAF antagonists, clearly implicate PAF as an important mediator of sepsis. The PAF antagonist BN52021, dosed in the mg kg^{-1} range, significantly reduces the mortality of septic patients with documented Gram-negative infections, but appears to be ineffective on Gram-positive infections[86]. The newer antagonists ABT299 (Ref. 87) and CL184005 (Ref. 88; Fig.7) appear to be 10–100-times more potent in animal models.

Strategies directed against tissue damage

Cytotoxic molecules released by activated neutrophils after their migration into target tissues cause tissue damage and organ failure. TNF-α and IL-1 can promote the expression of adhesion molecules on endothelial cells, such as ICAM-1, ELAM-1 and VCAM-1. Receptors on neutrophils and endothelial cells are involved in the margination, rolling and adherence of the neutrophils to the epithelium. This is followed by the transmigration of the neutrophilic granulocytes to inflammatory sites (liver and alveolar cavity), their adhesion to target tissues and further degranulation with release of destructive molecules such as superoxides, free radicals and enzymes. The inhibition of cell–cell interactions through adhesion molecules, involved in the adherence of neutrophils to the endothelium, or to their target tissue after transmigration can, thus, be expected to abrogate tissue damage. In this connection it has been shown that an anti-ICAM-1 antibody prevented LPS-induced acute lethality[89], and an anti-CD31 antibody reduced granulocyte-mediated hepatotoxicity induced by LPS (Ref. 90). Sulfated glycolipids (sulfatides), which are natural antagonists for L- and P-selectins, also prevented LPS-induced lethality in mice[91]. Thus, therapeutics directed against adhesion molecules are promising for prevention of organ dysfunctions such as hepatic failure and ARDS.

Agents that can inactivate the destructive molecules released during neutrophil degranulation (proteolytic enzymes, superoxides and free radicals) can also be used. The beneficial effects of some protease inhibitors have been mentioned above. A large panel of antioxidants has been tested and some of them might be of value[92]. In this regard, it has been shown very recently that treatment of rats with the new antioxidant U83836E reduced several LPS-induced effects including hypotension, serum TNF-α levels and lethality[93].

Conclusions and perspectives

In spite of considerable efforts made during several decades to develop strategies directed against Gram-negative sepsis, all the therapeutics proposed up to now, which first appeared promising in animals or in simplified human models, have been very disappointing when large and careful clinical trials were carried out. At the present time, none of these therapies can be considered as effective in clinical situations. This is very discouraging for pharmaceutical companies, and detrimental to patients for whom the prevalence of septic shock has been increasing, with an unacceptably high risk of death.

In the author's opinion, this failure is due mainly to basic gaps in our knowledge of LPS receptors, signaling pathways via these receptors and crosstalk between these pathways. In this regard, although the discovery of the LBP/CD14 signaling pathway has been a considerable conceptual progress in this field, its real importance in the pathology of endotoxic shock has probably been overestimated, whereas the role of co-receptors and other LPS receptors has not been sufficiently considered. Different observations indicate that the LBP/CD14 pathway is not specific for LPS and does not account for all of its effects:

- CD14, which was initially defined as a specific receptor for LPS, is now considered as a pattern-recognition receptor that can react with several components from Gram-negative and Gram-positive bacteria, and from yeast, such as peptidoglycan, lipoteichoic acid, lipoarabinomannan and various polysaccharides and amphiphilic molecules[94];
- CD14 is not necessarily required for responses to LPS because anti-CD14 antibodies do not abolish the responses of monocytes to high doses of LPS (Ref. 95), and because the LPS-induced production of acute phase proteins is not modified in CD14 knockout mice[96];
- In certain LPS-responsive cells, CD14 is neither constitutively expressed on the cell surface, nor involved in the responses. This has been clearly shown by Lichtman and colleagues in rat Kupffer cells[97], and by our group in bone marrow granulocytes[98];
- Crosstalk between LPS receptors and other receptors such as purinoceptors[70] or the PAF receptor[99] have been reported;
- Several molecules other than CD14 have been recognized as signaling LPS receptors (Table 1), including a low-affinity receptor sparsely expressed on bone marrow granulocytes[100], and the recently discovered toll-like receptor-2 expressed on all lymphoid tissues[9].

In spite of these recent advances, other LPS receptors remain unknown. Significant progress in the fight against Gram-negative sepsis presupposes that this gap in our knowledge is filled. Therefore, investigators should go back to basics and perform fundamental biochemical studies on LPS receptors and LPS signaling.

In addition to these fundamental studies, already explored therapeutic strategies must be improved by taking into account experienced pitfalls. Preventive therapies for patients at risk should preferentially make use of natural defence systems. Stimulation of neurohormonal mechanisms with neuropeptide analogs, and induction of tolerance to endotoxin with appropriate analogs of lipid A are potential candidates. However, if the inflammatory process is already initiated, the interruption of the pathogenic cascade at multiple sites by a cocktail of drugs can be expected to overcome the redundancy of the system and, thus, be much more effective than any single drug. Actually, the advantage of combined drug therapy has already been observed in an animal model[101].

In the author's opinion, there are three components that an active drug cocktail should have. The first is an early-active agent, such as a synthetic antagonist of LPS signaling, that is structurally related to lipid A and able to block both CD14-mediated and non-CD14-mediated signaling. PPDm2 and related structures are promising candidates. The second is an agent that acts at intermediate stages and is able to neutralize the release of large amounts of LPS. Optimal neutralization should associate binding, aggregation

Table 1. Cell surface LPS-binding molecules

LPS-induced signaling	Cell surface molecules	Cell types	Refs
No	CD18	Neutrophils, macrophages	102
	Scavenger receptor	Macrophages	103
	18, 25, 38, 40, 80 kDa proteins	Leukocytes	104
Yes	CD14	Myeloid cells	7
	CD11c	Macrophages, phagocytes	105
	Low-affinity receptor	Bone marrow granulocytes	100
	P-selectin	Platelets, neutrophils	106, 107
	216 kDa protein	Monocytes, astrocytes, endothelial, epithelial cells	108
	Toll-like receptor 2	Lymphoid tissues	9

and enzymatic deactivation of LPS. Enzymes that can deacylate or dephosphorylate the lipid A region of LPS could be linked to a potent LPS-binding molecule such as BPI, to give an efficient neutralizing hybrid molecule. The third component should intervene at later stages, by blocking the neutrophil degranulation and cellular damage that proceed from it. A compound acting at the level of the adhesion molecules of neutrophils, or an efficient antioxidant, can be used for this purpose. Given the marked interest in this field, we can be reasonably optimistic about devising adequate therapeutics in the near future.

REFERENCES

1 Medzhitov, R. and Janeway, J.A., Jr (1997) *Cell* 91, 295–298
2 Takayama, K. *et al.* (1983) *J. Biol. Chem.* 258, 7379–7385
3 Rietschel, E.T. and Brade, H. (1992) *Sci. Am.* 267, 54–61
4 Parillo, J.E. (1993) *New Engl. J. Med.* 328, 1471–1477
5 Taveira deSilva, A.M. *et al.* (1993) *New Engl. J. Med.* 328, 1457–1460
6 Tobias, P.S. *et al.* (1993) *J. Immunol.* 150, 3011–3021
7 Yu, B., Hailman, E. and Wright, S.D. (1997) *J. Clin. Invest.* 99, 315–324
8 Medzhitov, R., Preston-Hurlburt, P. and Janeway, C.A., Jr (1997) *Nature* 388, 394–397
9 Yang, R.B. *et al.* (1998) *Nature* 395, 284–288
10 Lu, B. *et al.* (1995) *J. Exp. Med.* 181, 2271–2275
11 Opal, S.M. and Yu, R.L., Jr (1998) *Drugs* 55, 497–508
12 Morrison, D.C. (1998) *Prog. Clin. Biol. Res.* 397, 199–207
13 Goldman, R. *et al.* (1987) *Nature* 329, 162–164
14 Onishi, H.R. *et al.* (1996) *Science* 274, 980–982
15 Treon, S.P., Thomas, P. and Broitman, S.A. (1993) *Proc. Soc. Exp. Biol. Med.* 202, 153–158
16 Read, T.E. *et al.* (1995) *Surgery* 117, 62–67
17 Vereschagin, E.I. *et al.* (1998) *Shock* 9, 193–198
18 Stegmayr, B.G. (1996) *Blood Purification* 14, 94–101
19 Sato, T., Orlowski, J.P. and Zborowski, M. (1993) *Asaio J.* 39, M790–M793
20 Donta, S.T. *et al.* (1996) *J. Infect. Dis.* 174, 537–543
21 Nys, M. *et al.* (1993) *Ann. Surg.* 217, 300–306
22 Bhattercharjee, A.K. *et al.* (1996) *J. Infect. Dis.* 173, 1157–1162
23 Cometta, A. *et al.* (1992) *New Engl. J. Med.* 327, 234–240
24 Girard, R. and Chaby, R. (1985) *Immunology* 56, 481–487
25 Pedron, T. *et al.* (1992) *Hybridoma* 11, 765–777
26 Cross, A.S. and Opal, S. (1994) *J. Endotox. Res.* 1, 57–69
27 Bouter, A.S. *et al.* (1994) *Serodiag. Immunother. Infect. Dis.* 6, 35–39
28 Kato, Y. *et al.* (1994) *Insect Biochem. Mol. Biol.* 24, 547–555
29 Warren, H.S. *et al.* (1992) *Infect. Immun.* 60, 2506–2513
30 Saladino, R. *et al.* (1994) *Circ. Shock* 42, 104–110
31 Matera, G. *et al.* (1993) *Antimicrob. Agents Chemother.* 37, 393–397
32 Schumann, R.R. *et al.* (1990) *Science* 249, 1429–1431
33 Selsted, M.E. *et al.* (1985) *J. Biol. Chem.* 260, 4579–4584
34 Lehrer, R.I., Lichtenstein, A.K. and Ganz, T. (1993) *Annu. Rev. Immunol.* 11, 105–128
35 Hirata, M. *et al.* (1993) *Infect. Immun.* 62, 1421–1426
36 Pereira, H.A. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 4733–4737
37 Weiss, J. *et al.* (1984) *J. Immunol.* 132, 3109–3115
38 Pajkrt, D. *et al.* (1996) *J. Exp. Med.* 184, 1601–1608
39 Wurfel, M.M., Hailman, E. and Wright, S.D. (1995) *J. Exp. Med.* 181, 1743–1754
40 de Haas, C.J.C. *et al.* (1998) *J. Immunol.* 161, 3607–3615
41 Kirikae, T. *et al.* (1998) *Infect. Immun.* 66, 1861–1868
42 Tobias, P.S., Mathison, J.C. and Ulevitch, R.J. (1988) *J. Biol. Chem.* 263, 13479–13481
43 Giroir, B.P. *et al.* (1997) *Lancet* 350, 1439–1443
44 Tobias, P.S. *et al.* (1997) *J. Biol. Chem.* 272, 18682–18685
45 Golenbock, T.D. *et al.* (1991) *J. Biol. Chem.* 266, 19490–19498
46 Ulmer, A.J. *et al.* (1992) *Infect. Immun.* 60, 5145–5152
47 Rudbach, J.A. *et al.* (1994) *Prog. Clin. Biol. Res.* 388, 107–124
48 Hofman, J. *et al.* (1994) *Prog. Clin. Biol. Res.* 388, 95–106
49 Tahri-Jouti, M.A. *et al.* (1990) *Mol. Immunol.* 27, 763–770
50 Charon, D. *et al.* (1998) *Bioorg. Med. Chem. Lett.* 6, 755–765
51 Pedron, T. *et al.* (1992) *Int. Immunol.* 4, 533–540
52 Sato, K. *et al.* (1995) *Infect. Immun.* 63, 2859–2866
53 Christ, W.J. *et al.* (1995) *Science* 268, 80–83
54 Kiani, A. *et al.* (1997) *Blood* 90, 1673–1683
55 Whalley, E.T. *et al.* (1992) *Agents Actions* 38 (Suppl.), 413–420
56 Eich-Rathfelder, S. *et al.* (1997) *Immunopharmacology* 36, 173–177
57 Siebeck, M. *et al.* (1992) *Agents Actions* 38 (Suppl.), 421–427
58 Uchiba, M. *et al.* (1997) *Am. J. Respir. Crit. Care Med.* 155, 711–718
59 Zohair, A. *et al.* (1989) *Biochem. J.* 257, 865–873
60 Quezado, Z.M. *et al.* (1994) *J. Exp. Med.* 179, 569–578
61 Hangen, D.H. *et al.* (1989) *J. Surg. Res.* 46, 195–199
62 Chow, C.W., Grinstein, S. and Rotstein, O.D. (1995) *New Horizons* 3, 342–351
63 Schütze, S. *et al.* (1992) *Cell* 71, 765–776
64 Inaba, H. and Filkins, J.P. (1991) *Am. J. Physiol.* 261, R26–R31
65 Ruetten, H. and Thiemermann, C. (1997) *Br. J. Pharmacol.* 122, 59–70
66 Badger, A.M. *et al.* (1996) *J. Pharmacol. Exp. Ther.* 279, 1453–1461
67 Hong, H.J., Wu, C.C. and Yen, M.H. (1998) *Clin. Exp. Pharmacol. Physiol.* 25, 600–606
68 Yu, S.M. *et al.* (1997) *Biochem. J.* 328, 363–369
69 Zabel, P. *et al.* (1989) *Lancet* 2, 1474–1477
70 Merten, M.D., Kammouni, W. and Figarella, C. (1995) *FEBS Lett.* 369, 202–206
71 Proctor, R.A. *et al.* (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 6017–6020
72 Parmely, M.J. *et al.* (1993) *J. Immunol.* 151, 389–396
73 Firestein, G.S. *et al.* (1994) *J. Immunol.* 152, 5853–5859

74 Tschaikowsky, K., Schmidt, J. and Meisner, M. (1998) *J. Pharmacol. Exp. Ther.* 285, 800–804
75 Rice, G.C. *et al.* (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 3857–3861
76 Gillard, J. *et al.* (1989) *Can. J. Physiol. Pharmacol.* 67, 456–464
77 Bone, R.C. *et al.* (1987) *New Engl. J. Med.* 317, 653–658
78 Abou-Mohamed, G. *et al.* (1995) *J. Pharmacol. Exp. Ther.* 275, 584–591
79 Alexander, H.R. *et al.* (1991) *Infect. Immun.* 59, 3889–3894
80 Kilbourn, R.G. *et al.* (1990) *Biochem. Biophys. Res. Commun.* 172, 1132–1138
81 Macmicking, J.D. *et al.* (1995) *Cell* 81, 641–650
82 Aldeken, K.J. *et al.* (1998) *Shock* 9, 289–295
83 Rees, D.D., Monkhouse, J.E. and Moncada, S. (1995) *Intensive Care Med.* 21, 521
84 Perrella, M.A. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 2054–2059
85 Bune, A.J. *et al.* (1996) *Biochem. Biophys. Res. Commun.* 220, 13–19
86 Tenaillon, A. *et al.* (1993) *Am. Rev. Respir. Dis.* 147, A97
87 Curtin, M.L. *et al.* (1998) *J. Med. Chem.* 41, 74–95
88 Torley, L.W. *et al.* (1992) *Antimicrob. Agents Chemother.* 36, 1971–1977
89 Watanabe, S. *et al.* (1995) *Int. Immunol.* 7, 1037–1046
90 Sauer, A. *et al.* (1996) *J. Leukocyte Biol.* 60, 633–643
91 Higashi, H. *et al.* (1997) *Infect. Immun.* 65, 1223–1227
92 Schiller, H.J., Reilly, P.M. and Bulkley, G.B. (1993) *Crit. Care Med.* 21, S92–S102
93 Altavilla, D. *et al.* (1998) *Br. J. Pharmacol.* 124, 1293–1299
94 Pugin, J. *et al.* (1994) *Immunity* 1, 509–516
95 Lynn, W.A., Liu, Y. and Golenbock, D.T. (1993) *Infect. Immun.* 61, 4452–4461
96 Haziot, A. *et al.* (1998) *J. Immunol.* 160, 2570–2572
97 Lichtman, S.N., Wang, J. and Lemasters, J.J. (1998) *Am. J. Physiol.* 275, G39–G46
98 Pedron, T. *et al.* (1994) *J. Biol. Chem.* 269, 2426–2432
99 Waga, I. *et al.* (1993) *Biochem. Biophys. Res. Commun.* 197, 465–472
100 Girard, R., Pedron, T. and Chaby, R. (1997) *Immunology* 91, 391–398
101 Naess, F. *et al.* (1992) *Eur. J. Surg.* 158, 95–103
102 Wright, S.D. *et al.* (1989) *J. Exp. Med* 169, 175–183
103 Hampton, R.Y. *et al.* (1991) *Nature* 352, 342–344
104 Lei, M.G. and Morrison, D.C. (1990) *Int. Rev. Immunol.* 6, 223–235
105 Ingalls, R.R. and Golenbock, D.T. (1995) *J. Exp. Med.* 181, 1473–1479
106 Malhotra, R. *et al.* (1998) *Eur. J. Immunol.* 28, 983–988
107 May, G.L. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 183, 1062–1069
108 Vita, N. *et al.* (1997) *J. Immunol.* 158, 3457–3462

In short…

A new report produced by Datamonitor for Reuters Business Insight reveals the increasing importance of alliances, rather than acquisitions, between biotechnology companies and major pharmaceutical firms in the effort to improve R&D productivity.

According to the report, *Integrating genomics, the next generation*, the rising cost of R&D is now a major concern within the pharmaceutical industry, and the exploitation of key enabling technologies, including genomics, will help to provide an answer to this problem and allow for growth in the next decade.

Key findings of the report include:

- the value of biotechnology alliances will waver as pharmaceutical companies look to tighten their R&D expenditures;
- consolidation is likely to occur among genomics companies;
- genetic diagnostic tests are likely to be some of the first products generated by genomics.

A major finding of the report is that, in the drive to rapidly discover new drugs, pharmaceutical companies are looking to initiate large numbers of alliances with a range of specialist biotechnology companies. The report claims that alliances offer pharmaceutical companies the opportunity to 'tap into' established expertise in key areas such as genomics, whilst also maintaining flexible arrangements. Of these alliances, the report found that many of the new deals involve 3–5 year collaborations with pharmaceutical companies increasingly including 'get out' clauses in the contracts, allowing them to terminate the agreement early if the project proves unsuccessful and thus enabling them to refocus their resources elsewhere.

The report also points out, however, that the value of these deals is likely to decrease, as pharmaceutical companies become more selective in their choice of partners and the technologies that they are accessing. The report also highlighted the fact that, in the future, the perceived success of biotechnology firms may be increasingly based on the ability of the company to form alliances with major pharmaceutical players.

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