REVIEW ARTICLE

Bacterial endotoxin and current concepts in the diagnosis and treatment of endotoxaemia

KATHERINE F. BAYSTON and J. COHEN

Infectious Diseases Unit, Departments of Bacteriology and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN

Background

The study of endotoxin began at the end of the 19th century when Richard Pfeiffer, a pupil of Robert Koch, found that lysates of heat-inactivated *Vibrio cholerae* contained a toxic principle which was capable of inducing shock and death in experimental animals. He termed this heat-stable toxin "endotoxin" to distinguish it from the heat-labile exotoxins which were actively secreted by live *V. cholerae*. Around the same time two other scientists, Eugenio Centanni and Hans Buchner, independently isolated the same toxin. Centanni made two important contributions. First, he observed that this toxin could be isolated from lysates of many different gram-negative bacteria, but never from similar preparations of gram-positive bacteria. Second, he drew attention to the remarkable pyrogenic properties of endotoxin. Buchner was the first to demonstrate the association of endotoxin with leucocytosis and altered host immunity.

It was not until 1935 that Boivin and Messrobianu, using a method of trichloroacetic extraction, determined that the endotoxic activity of gram-negative bacterial lysates resided in an outer-membrane macromolecular complex of protein, lipid and polysaccharide. Two decades later Westphal and Luderitz commenced their classic studies on the biochemistry of endotoxin. Protein-free lipopolysaccharide (LPS), prepared by phenol-water extraction and purified, possessed all the properties of crude endotoxin. Further separation of LPS into a water-soluble polysaccharide fraction and a chloroform-soluble lipid fraction led to the finding that the biological activity of endotoxin resided in the lipid moiety, now termed Lipid A. More recently Lipid A has been chemically synthesised and both natural and synthetic molecules display identical activities.

Now that the chemical structure of endotoxin has been elucidated, current research focuses on delineating the mechanisms of action of endotoxin, developing simple but sensitive systems of endotoxin detection, and identifying potential methods of treating endotoxin-related disease. This review aims to give the reader an overview of the subject with particular emphasis on clinically related aspects.

Structure and chemical nature of endotoxin

A detailed knowledge of the biochemistry of the cell wall of gram-negative bacteria is helpful in understanding the structural basis of the toxicity and also the rationale for the recent approaches to the treatment of endotoxic shock.

The cell wall of gram-negative bacteria is a complex structure consisting of the innermost cytoplasmic membrane, the periplasm, the peptidoglycan layer, the outer membrane and, in many instances, additional structures such as capsules, extracellular polysaccharide, fimbriae and flagella (fig. 1). Endotoxin (LPS) is found exclusively in the outer membrane and, specifically, only in the outer leaflet of this membrane. Here LPS forms a hydrophobic barrier which restricts the entry of noxious substances such as bile salts, digestive enzymes and certain antibiotics, and enables the bacterium to evade many innate host-defence factors including complement, lysozyme and cationic proteins.^{1,2} Endotoxin may also be found in a cell-free form occurring after bacterial autolysis, as a result of exposure to cell-membrane toxins or antibiotics, during rapid (log-phase) growth, or when essential nutrients are depleted from the environment—all of these conditions may arise during septicæmia.^{3–5}

The molecular structure of LPS has been investigated in great detail.^{6,7} Three well-defined regions

Fig. 1. Schematic representation of gram-negative bacterial cell wall.

(fig. 2) can be demonstrated: (i) an O-specific side chain; (ii) core oligosaccharide; and (iii) Lipid A. The O-specific side chain consists of repeating oligosaccharide units, each composed of two to six monosaccharides. Within these units are distinct carbohydrate residues which exhibit unique antigenic properties. These antigenic determinants, or O-factors, determine the serotype of a given bacterial species and, hence, there are many hundreds of variations. The O chain is the most immunogenic region of endotoxin and gives rise to

the production of specific anti-O antibodies which, in animal models of infection, are able to confer a high degree of protection.^{8,9}

The core region is a branching oligosaccharide consisting of several common, and two unusual, sugars which are added sequentially by unique glycosyl transferases. The Lipid A-distal outer core contains frequently occurring sugars such as glucose, galactose and N-acetylglucosamine, whilst the Lipid A-proximal inner core is composed of heptose and keto-deoxyoctonate (KDO), usually

Fig. 2. General structure of salmonella LPS showing: A-D, sugar residues; Glc, D-glucose; Gal, D-galactose; GlcN, N-acetyl-D-glucosamine; Hep, L-glycero-D-manno-heptose; KDO, 2-keto-3-deoxymanno-octonate; AraN, 4-amino-L-arabinose; P, phosphate; EtN, ethanolamine; ~ hydroxy- and non-hydroxy fatty acids. Ra-Re are incomplete R-form lipopolysaccharides. (Reproduced with permission from B. J. Appelmeik).

carrying charged residues such as phosphate and phosphorylethanolamine. Although the core oligosaccharide shows some structural variability, this is minor in comparison with that of the O chain, only six or seven variations existing amongst the common Enterobacteriaceae. Several bacterial mutant strains have been isolated in which the O chain and part of the core oligosaccharide are lacking as a result of a defect in the biosynthesis of the core region. Because of their colonial morphology such mutant strains are called rough (in contrast to the smooth colonies of bacteria possessing O antigen). Depending on the site of the defect, these strains are labelled Ra-Re (fig. 2). Rough mutant strains, in particular the J5 (Rc) mutant strain of *Escherichia coli* O111 and the Re strain 595 of *Salmonella minnesota*, have been used extensively as antigenic stimuli for the production of anti-core antibodies. The theoretical advantage of these antibodies is that, because they are raised against epitopes common to many different lipopolysaccharides, they should provide cross-protection against a wide variety of gram-negative bacteria. This has been difficult to prove, however, and there are probably as many studies showing lack of cross-protection as there are demonstrating its presence (*vide infra*).

Recent studies of non-enteric gram-negative pathogens, including *Haemophilus influenzae*, *Neisseria meningitidis*, *N. gonorrhoeae* and *Bordetella pertussis*, have shown that these organisms, like rough mutant strains, also lack O-antigens. Although more heterogeneity is found in the core polysaccharide component of LPS in these bacteria, the general structure is otherwise similar to that occurring in enteric organisms.^{10,11}

Lipid A, the most highly conserved part of LPS, consists of a phosphorylated glucosamine-disaccharide backbone to which long-chain fatty acids are bound. It is linked to the core region by a ketosidic bond to KDO. This bond is extremely sensitive to acid hydrolysis and, as a result, Lipid A may readily be dissociated from the O chain and core-polysaccharide components of LPS. In soluble form, free Lipid A possesses most of the endotoxic properties of intact LPS.¹² Synthetic Lipid A has recently been shown to possess activity identical to that of native Lipid A.¹³ The full spectrum of endotoxic activity appears to require the presence of a disaccharide, two phosphoryl groups and minimally five, but ideally six, acyl residues in a defined distribution. Small changes in the structure of Lipid A (native or synthetic) can lead to a marked reduction in its toxicity. For example, monosaccharide and monophosphoryl precursors of Lipid A, such as Lipid X and monophosphoryl

Lipid A (MPL), lack the ability to induce the typical in-vivo toxicity of endotoxin.^{14,15} For unknown reasons, however, other biological effects of these precursors, including the stimulation of cytokine release, the induction of tolerance to toxic forms of Lipid A, and the ability to cause gelation of the *Limulus* amoebocyte lysate (*vide infra*) are preserved.

Clinical associations

Endotoxin has been implicated in the pathogenesis of a variety of different clinical disorders (table). Of these, gram-negative septic shock is the most familiar, and is the setting in which the role of endotoxin is most clearly established.¹⁶ Gram-negative septicaemia is a condition which is increasing in incidence, largely as a result of the greater use of invasive medical procedures and immunosuppressive agents. The mortality associated with gram-negative bacteraemia is high, estimated figures varying from 20 to 50%. Several factors have been shown to influence this; e.g., age, underlying disease and the appropriate use of antibiotics.¹⁷ The greatest prognostic factor, however, is the development of shock.¹⁸ Septic shock is a syndrome characterised by hypotension, oliguria, hypoxia, acidosis, the development of microvascular abnormalities, and disseminated intravascular coagulation.¹⁹ Multiple organ failure is an all-too-common sequel. Studies at necropsy reveal widespread tissue damage with particular involvement of the liver, lungs, kidneys and adrenal glands.

Table. Some clinical conditions in which endotoxin has been implicated*

Disease association	Key reference
Septic shock	see text
Liver disease:	
fulminant hepatic failure	35
cirrhosis	81
renal failure in obstructive jaundice	34
Inflammatory bowel disease	27
Acute renal failure	80
Glomerulonephritis	37
Adult respiratory-distress syndrome	30
Major abdominal trauma	29
Neonatal necrotising enterocolitis	33
Radiation injury	82
Graft-versus-host disease	36
Toxic-shock syndrome	83

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Tissue lesions include oedema, haemorrhage, inflammatory infiltrates, fibrin thrombi and areas of tissue necrosis. Identical physiological and pathological changes may be seen in experimental animals receiving lethal doses of endotoxin.

Shock is estimated to occur in about 20–40% of patients with gram-negative septicaemia. Of these, c. 75% die despite the use of potent antibiotics and intensive-care facilities. One explanation for this is that, whilst antibiotics are very effective at killing bacteria, they have no activity against endotoxin or, indeed, the host-derived factors which are now thought to mediate the toxic effects of endotoxin (*vide infra*). It is of interest that other organisms including gram-positive bacteria, spirochaetes, rickettsiae, mycoplasmas, parasites, fungi and viruses may also cause a shock-like syndrome indistinguishable from that induced by gram-negative bacteria. Recent evidence indicates that, whilst endotoxin is perhaps the most potent stimulus to mediator production, factors derived from other organisms may also do so.^{20, 21} It seems, therefore, that regardless of aetiology, a final common pathway is involved in the production of septic shock.

In comparison with the huge volume of literature on endotoxic shock, there is considerably less data concerning the role of endotoxin in the aetiology of other conditions. There are, however, a number of clinical and experimental observations supporting the theory that endotoxin derived from bacteria in the gut may leak into the circulation in the absence of septicaemia and thus contribute to the pathogenesis of other diseases.²² Endotoxin is usually present in large quantities in the human gut without producing harmful effects. Even the ingestion of milligram quantities of endotoxin fails to produce adverse reactions in healthy human volunteers. This is largely because the gut mucosa of healthy individuals is both impervious to and resistant to the effects of intestinal endotoxins. There are three routes by which endotoxaemia may occur: *via* the portal vein, by direct transmural absorption into the systemic blood stream, or by the intestinal lymphatics. Of these, the first two routes are considered the most important in man. If the gastrointestinal mucosa, portal circulation and hepatic-reticuloendothelial system are intact, gut-derived endotoxin does not appear to give rise to detectable systemic endotoxaemia. In contrast, several animal models have revealed that if the gut is damaged, e.g., by an acute ischaemic event, after the induction of inflammatory bowel disease, or after total-body irradiation, endotoxins will trans-migrate through the bowel mucosa.^{23–25} Again,

experimental acute portal-vein occlusion also gives rise to portal and systemic endotoxaemia.²⁶ Several clinical studies support these findings. Marked endotoxaemia was found in 12 patients suffering from severe relapse of inflammatory bowel disease.²⁷ In this study whole-gut irrigation, used as a means of reducing the gut pool of endotoxin, resulted in a significant decline in circulating endotoxin, normalisation of temperature in all seven febrile patients and a fall in ESR. Similar findings have been documented by other investigators, e.g., it has been shown that systemic endotoxaemia is of common occurrence in patients with inflammatory bowel disease undergoing abdominal surgery.²⁸ Major trauma patients frequently exhibit signs and symptoms of septic shock in the absence of documented septicaemia and evidence suggests that in these patients damage to the gastrointestinal mucosa or the reticuloendothelial system may lead to endotoxaemia and many of its clinical sequelae.²⁹ In particular, endotoxin has been implicated in the aetiology of adult respiratory distress syndrome (ARDS), a syndrome with a high mortality and which may complicate the course of major trauma victims as well as that of patients suffering from a variety of other serious disorders.³⁰ Fever, with or without the manifestations of septic shock, is of almost universal occurrence in patients who are neutropenic after chemotherapy or irradiation; less than half of these episodes have a microbiologically documented cause. Endotoxaemia arising from the intestinal tract is thought by some to play a role in the aetiology of many of these otherwise unexplained episodes of fever,³¹ although not all workers agree.³² Other situations in which gut-derived endotoxin has been implicated are fever and thrombocytopenia associated with neonatal necrotising enterocolitis,³³ acute renal failure in patients with obstructive jaundice,³⁴ and the development of fulminant hepatic failure in patients with cirrhosis of the liver.³⁵ Last, intestinal endotoxin may potentiate immunologically-mediated processes such as graft-*versus*-host disease³⁶ and antibody-mediated nephritis.³⁷

Biological effects

Much published data based on in-vitro and in-vivo studies testify to the biological havoc that endotoxin can cause. It exerts a profound influence on the formed elements of the blood and, in particular, on the coagulation system, causes metabolic and pharmacologic effects which account in large part for the pathophysiological changes seen in shocked patients, and is a powerful immuno-

stimulant with the ability to influence both cellular and humoral limbs of the immune response. These topics have been the subject of several recent reviews to which the reader is referred for more information.^{16, 38, 39}

Much, if not all, of the toxicity of endotoxin is brought about by a series of mediators rather than by endotoxin itself. Several of these have been recognised for some years—the anaphylatoxins C3a and C5a, arachidonic-acid derivatives, reactive oxygen intermediates, endorphins, coagulation factors and platelet-activating factor. Other more recently described procoagulant molecules, such as tissue factor and intercellular adhesion-molecule 1 (ICAM 1), probably also contribute to the development of shock and, in particular, disseminated intravascular coagulation. What has caused most interest in the field of endotoxin research in recent years, however, is the discovery of the role of cytokines in septic shock. Tumour-necrosis factor α (TNF), interferon γ (IFN- γ), interleukin 1 (IL-1), interleukin 2 (IL-2), and more recently interleukin 6 (IL-6) have all been incriminated. Of these, TNF has received most attention.

Macrophages are the principal source of endotoxin-induced mediators, including TNF. This cytokine was first identified as a factor which caused a reduction in lipoprotein-lipase activity and seemed to be responsible for the markedly lipaemic serum found in cachectic animals in the advanced stages of trypanosomiasis.⁴⁰ Kawakami and Cerami noted that suppression of lipoprotein lipase also followed endotoxin administration to endotoxin-sensitive mice, but not to an endotoxin-resistant (C3H/HeJ) mouse strain.⁴¹ The cellular source of this factor was found to be the macrophage, and endotoxin proved an extremely potent stimulus for the release of TNF from macrophages of endotoxin-sensitive mice. Studies with purified material showed that TNF was pyrogenic and that, when infused into animals, it produced all the clinical and pathological features of septic shock.⁴² Passive immunisation against TNF was shown to protect mice⁴³ and rabbits⁴⁴ against the lethal effects of purified endotoxin and, later on, baboons⁴⁵ against *E. coli* septicaemia. Protection was associated with markedly reduced levels of circulating TNF.

In man, as well as in animals, there is growing evidence of a role for TNF in septic shock.⁴⁶ Human monocytes, like murine macrophages, produce large amounts of TNF *in vitro* in response to stimulation with LPS. Mitchie, in a study of 11 male volunteers given endotoxin, demonstrated that plasma levels of TNF increased 90–180 min

after endotoxin administration and that this coincided with the development of flu-like symptoms, fever, tachycardia, increased circulating levels of stress hormones and changes in the peripheral white-cell count.⁴⁷ Cancer patients treated with recombinant TNF developed features of septic shock, notably fever, hypotension, abnormal liver enzymes, leucopenia and renal impairment.⁴⁸ Finally, several groups reported the presence of circulating TNF in patients with septicaemia. In those with systemic meningococcal disease, high serum levels of TNF were associated with a poor outcome.^{49, 50} Several groups are currently studying the therapeutic efficacy of anti-TNF antibody in patients with septic shock but results have not yet been published.

Considered overall, these results led many investigators to conclude that TNF was probably the principal, if not the sole, mediator of endotoxicity. However, it now appears that this is a considerable oversimplification of the truth. Several recent studies have demonstrated that TNF alone is insufficient to induce septic shock and that, by implication, other mediators must be required. Rothstein and Schreiber showed that the administration of endotoxin-free recombinant TNF to pathogen-free mice lacked toxicity unless small amounts of endotoxin or other microbial agents were added.⁵¹ Kiener *et al.* gave MPL, a non-toxic derivative of Lipid A, to mice and showed that, although similar levels of TNF were induced in mice receiving MPL, these animals survived whilst those challenged with Lipid A died.¹⁵ Similarly, in our own studies, clone 20, a monoclonal anti-core-endotoxin antibody, protected mice from an LD90 dose of *E. coli* yet TNF levels were no different from those of control mice.⁵²

In summary, endotoxin triggers a series of cytokine and non-cytokine mediators, many of which have overlapping or synergic effects and many of which may themselves induce the production of another mediator. Undoubtedly, some, such as TNF, have a more critical role than others, but no single mediator is solely responsible for all the manifestations of septic shock.

Sources of endotoxin

Endotoxin is not detectable in the circulation in healthy individuals, and so how, and under what circumstances, may it appear? The most obvious association is gram-negative septicaemia although, as we have seen, endotoxaemia and bacteraemia are not always synonymous. Gram-negative bacilli in culture will liberate endotoxin into the supernate

to varying degrees; indeed, in the case of meningococci, it appears that the extent of endotoxin release correlates with virulence.⁵³ Spontaneous endotoxin release may well occur *in vivo* during bacteraemia, perhaps reflecting cell division and multiplication. It is also not difficult to imagine that endotoxin might seep into the circulation from an enclosed abscess, even if bacteraemia was absent. An additional major source of endotoxin is the gut, and it is clear that damage to the mucosal barrier may lead to endotoxaemia independently of bacteraemia.²²

A more intriguing possibility is that endotoxin may be released into the circulation as a result of bacterial lysis, either complement-mediated or as a result of antibiotic administration. Since most bacteraemic strains of gram-negative bacteria are serum resistant,⁵⁴ we must assume that antibody- and complement-mediated killing is not an important source of endotoxin in septicemia. It is more difficult to judge the potential importance of antibiotic-mediated endotoxin release. Clinical observations dating back to the 1940s had led to the suggestion that the sudden lysis of bacteria, when first exposed to antibiotic, might cause endotoxin release and symptoms of shock. We and others have shown that *in-vitro* exposure of bacteria to certain classes of bactericidal antibiotics causes significant amounts of endotoxin to be released promptly into the supernate.⁵⁵ What has been more difficult (and is of greater importance) is to try and establish if this phenomenon is of significance *in vivo*. Animal studies have produced conflicting results: in a rabbit model of *E. coli* sepsis, there was no correlation between endotoxin levels and survival.⁵⁶ In contrast, Rokke has more recently reported a study in piglets in which gentamicin-induced endotoxin release was clearly associated with adverse effects on cardiac output and pulmonary-artery pressures.⁵⁷ Perhaps the best evidence comes from a model of gram-negative meningitis in rabbits, in which antibiotic treatment caused a

rise in CSF endotoxin and an associated increase in cerebral oedema.⁵⁸ Unfortunately, this is a difficult area in which to do clinical studies and, whilst some groups have found evidence of endotoxin release, others have not.

Measurement of endotoxin

The most widely used and most sensitive method of detecting endotoxin is the *Limulus* amoebocyte-lysate (LAL) assay.^{59,60} This assay was developed after a chance discovery that the horseshoe crab, *Limulus polyphemus*, developed disseminated intravascular coagulation upon infection with gram-negative bacteria. Subsequent studies revealed that clotting of the *Limulus* haemolymph was caused by a particular component of the bacteria, namely endotoxin, and that picogram quantities of purified endotoxin were sufficient to induce this effect. All the factors necessary for activation of the clotting process could be found within granules present in specialised blood cells called amoebocytes. With a lysate of these cells, a simple, gel-clot test for the detection of endotoxin was devised. The principle of this test is that gelation occurs when a sample containing endotoxin causes activation of a series of primitive enzymes present in the lysate, a process somewhat analogous to the human coagulation cascade. Since the rate of gelation of the lysate is dependent on the amount of endotoxin present, the assay is semi-quantitative.

In addition to the gel-clot method, several other methods for the detection of endotoxin have been based on LPS-induced LAL activation: turbidometric and nephelometric measurements of the gelation reaction; determination of the protein content of the gel clot; rocket immunoelectrophoresis; and direct measurement of the action of activated clotting enzyme on a synthetic chromogenic substrate (fig. 3). This latter method is a fully quantitative micro-assay which can be performed

Fig. 3. Principle of the *Limulus* amoebocyte-lysate (LAL) test in which gel formation or colour production may be used as an endpoint. (Reproduced from Cohen,⁸⁴ with permission from the publisher).

in a standard 96-well microtitration plate and the colour production then recorded on an automatic ELISA plate-reader.⁶¹ As little as 5–10 pg of endotoxin may be detected with this version of the LAL assay. Kits containing all the necessary reagents and standards are available commercially; however, as with most bioassays, there are a number of pitfalls in the use of the endotoxin assay that may limit its application.

The specificity of the LAL reaction for endotoxin has been questioned by several investigators. Certain products of gram-positive bacteria and fungi may react with LAL if present in very high concentrations. Thrombin, thromboplastin, RNAases, calcium gluconate and certain synthetic polysaccharides have also been found to cause LAL gelation. Fortunately, the chances of these substances being present in sufficient quantity in clinical samples to affect the LAL assay are unlikely. By far the commonest cause of false-positive reactions is endotoxin contamination from the environment. If false-positive results are to be avoided, the assay must be performed with utmost care and all materials and reagents should be pyrogen-free.

Perhaps the major impediment to the successful clinical application of the LAL assay is the presence of inhibitors in serum and plasma. Recovery of endotoxin from serum is low and somewhat erratic because of entrapment of LPS within the fibrin network during clotting.⁶² For this reason, most investigators use plasma, adding the minimum possible amount of heparin (c. 10 units/ml) to prevent blood clotting, as heparin and other anticoagulants are themselves inactivators of the LAL-coagulation cascade. Despite the use of plasma, recovery of endotoxin remains poor unless procedures are used to overcome the effect of other inhibitors. LPS binds to naturally occurring anti-LPS antibodies, high-density lipoproteins, anti-thrombin III, α -2-macroglobulin, as well as to other poorly characterised plasma proteins. Several methods have been developed to overcome the effect of these inhibitors: (i) chloroform extraction; (ii) acidification or alkalisation procedures; (iii) perchlorate extraction; (iv) gel filtration; and (v) diluting and heating. Of these, dilution and heating methods are the simplest and have been adopted almost universally as the most convenient and effective means of removing inhibitors. In addition, it should be noted that changes in pH, as well as in the concentrations of calcium and magnesium, may affect the assay and that different batches of *Limulus* lysate may vary in sensitivity to endotoxin.

For all the reasons detailed above, the LAL assay

is not well suited as a routine diagnostic test; its principal use is in the testing of parenteral fluids, biological materials and medical devices by pharmaceutical companies. That said, there are clinical situations in which it may be extremely useful. The most obvious example is in meningitis caused by gram-negative bacilli, particularly in neonates. CSF is largely free of the technical problems associated with plasma and a small sample, carefully taken, may be tested for endotoxin and a result obtained within 2–3 h. In cases in which a direct smear is negative, or if the patient has already received antibiotics, a positive result may be extremely valuable.⁶³ Other situations, in which it has been suggested that the assay might have a clinical application, include gonorrhoea, bacteriuria and CAPD infections.^{64–66}

The greatest debate surrounds the use of the LAL assay in the diagnosis and management of suspected gram-negative septicæmia. Early results suggested that the presence and degree of endotoxaemia correlated with outcome.⁶⁷ Results of later studies were not so optimistic and, in a review of 17 studies published between 1970 and 1979, Elin concluded that the assay lacked sufficient sensitivity and specificity to be clinically useful.⁶⁸ Since the 1970s, the assay has undergone considerable refinement. A further review by Elin of 10 studies published between 1976 and 1984 showed that sensitivity, specificity and predictive value had all increased.⁶⁹ Despite these improvements, the use of the LAL assay for the rapid diagnosis of gram-negative septicæmia remains unsatisfactory. It has become apparent that endotoxin is not always present in the serum of patients with gram-negative septicæmia and, conversely, that conditions other than sepsis may result in endotoxaemia.²² Currently, the most promising use of the LAL assay in septicæmia is as a guide to prognosis. Indeed, in a recent study by Brandtzaeg *et al.*⁷⁰ of 45 patients with systemic meningococcal disease, it was shown that initial plasma-endotoxin levels of <25, 25–700, 700–10 000 and >10 000 pg/ml were associated with 0%, 14%, 27% and 86% fatality levels, respectively. Endotoxin levels >700 pg/ml correlated with the development of shock ($p < 0.0001$). Because endotoxin from different organisms will activate the LAL assay to differing degrees, it is particularly important to be aware that quantitative measurements such as these need careful interpretation.

Implications for treatment

The high mortality of septic shock, despite antibiotic treatment, indicates the need for addi-

tional therapeutic options. Attempts to neutralise the effects of endotoxin appear the most promising of the approaches investigated to date. Two strategies have been adopted: (i) the use of antibodies directed against endotoxin itself; and (ii) antibodies targetted against mediators of endotoxin. In this regard, anti-core-endotoxin antibodies and anti-TNF antibodies have received most attention.

Anti-core-endotoxin antiserum was first used in animal studies more than 20 years ago. The rationale for its subsequent evaluation in man came from the following experimental observations. Polyclonal antiserum raised against rough mutant bacteria was found to protect against the toxic sequelae of endotoxin in a wide variety of animal models.⁷¹ Further studies showed that this protective effect could also be demonstrated against live bacterial infection and, moreover, that protection extended to heterologous as well as homologous strains.⁷² Antibody prophylaxis was effective both in endotoxin-sensitive species, such as rabbits, and in animals of lesser sensitivity, such as mice.^{72,73} Finally, the discovery of naturally occurring antibodies to endotoxin-core structures, in normal animals as well as in man, led to a series of retrospective studies in patients with gram-negative bacteraemia, that demonstrated that patients with high levels of anti-core antibody at the time of presentation had a reduced incidence of septic shock and a lower mortality.^{8,74} Based on this evidence, Ziegler *et al.* commenced a multicentre, double-blind clinical trial of polyclonal anti-J5 antiserum *versus* control (non-immune) serum in patients with septic shock.⁷⁵ Mortality was reduced overall from 39% in control patients to 22% in recipients of anti-J5 antiserum (p 0.011). In the subgroup of patients with severe shock, the results were even more striking; 77% of control patients died compared with 44% of the anti-J5 group (p

0.003). A subsequent study by Baumgartner *et al.*⁷⁶ investigated the prophylactic use of anti-J5 antiserum in high-risk intensive-care patients. Although the incidence of bacteraemia was the same in control and anti-J5 groups, anti-J5 protected significantly against the development of shock.

Unfortunately these results represent only half the story: many in-vitro studies have failed to show cross-reactivity and many in-vivo studies cannot demonstrate protection. Even in those studies showing protection, it has been impossible to prove that this is specifically antibody-mediated, and not due to small amounts of contaminating endotoxin, which might cause tolerance,⁷⁷ or to polyclonal B-cell activation,⁷⁸ or to other non-antibody proteins present in the antiserum.⁷⁹ It is hoped that, in due course, studies with monoclonal antibodies will clarify this issue. The results of three such clinical trials are eagerly awaited.

A more recent approach has been the development of antibodies to mediators and, in particular, to TNF. To date, there has been uniform agreement on the efficacy of anti-TNF given as prophylaxis in experimental models of gram-negative septic shock.⁴³⁻⁴⁵ The number of reported studies is small, however, and clinical trials have not yet been reported. A major concern is the apparent lack of protection if antibody is given after, rather than before, bacterial challenge. A potential advantage is that anti-TNF may also be effective in the management of shock caused by organisms other than gram-negative bacteria.

It is evident that much work remains to be done, but it is hoped that in the next few years immunotherapy will emerge as a useful adjunct to the management of patients with septic shock.

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