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

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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 Messro-beanu, using a method of trichloracetic 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. 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 septicaemia.

The molecular structure of LPS has been investigated in great detail. Three well-defined regions...
Fig. 1. Schematic representation of gram-negative bacterial cell wall.

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-deoxyoctonate; 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. Appelmelk.)
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 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 (Re) 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 (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 septicemia 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 bacteremia 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>
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* Reproduced from Cohen84 with permission from the publisher.
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 transmigrate 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, 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 disease\(^{36}\) 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.\textsuperscript{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 \( \alpha \) (TNF), interferon \( \gamma \) (IFN-\( \gamma \)), 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{42} Passive immunisation against TNF was shown to protect mice\textsuperscript{43} and rabbits\textsuperscript{44} against the lethal effects of purified endotoxin and, later on, baboons\textsuperscript{45} against \textit{E. coli} septicicaemia. 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.\textsuperscript{46} Human monocytes, like murine macrophages, produce large amounts of TNF \textit{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.\textsuperscript{47} Cancer patients treated with recombinant TNF developed features of septic shock, notably fever, hypotension, abnormal liver enzymes, leucopenia and renal impairment.\textsuperscript{48} Finally, several groups reported the presence of circulating TNF in patients with septicemia. In those with systemic meningococcal disease, high serum levels of TNF were associated with a poor outcome.\textsuperscript{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.\textsuperscript{51} Kiener \textit{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.\textsuperscript{15} Similarly, in our own studies, clone 20, a monoclonal anti-core-endotoxin antibody, protected mice from an LD90 dose of \textit{E. coli} yet TNF levels were no different from those of control mice.\textsuperscript{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 septicemia 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 \textit{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 septicaemia. 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 \textit{in vivo}. Animal studies have produced conflicting results: in a rabbit model of \textit{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, \textit{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.

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**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 microtitre plate and the
colour production then recorded on an automatic
ELISA plate-reader.\(^6\) 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, RNAses, calcium gluconate and certain synthetic
polysaccharides have also been found to cause LAL
gelation. Fortunately, the chances of these sub-
stances 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.\(^6\) 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, \(\alpha\)-2-macroglobulin, as well as to other
poorly characterised plasma proteins. Several meth-
ods have been developed to overcome the effect of
these inhibitors: (i) chloroform extraction; (ii)
acidification or alkalinisation 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
lysat 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 phar-
maceutical 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.\(^6\) Other situations, in which it has been
suggested that the assay might have a clinical
application, include gonorrhoea, bacteriuria and
CAPD infections.\(^6\)–\(^6\)

The greatest debate surrounds the use of the LAL
assay in the diagnosis and management of suspected
gram-negative septicaemia. Early results suggested
that the presence and degree of endotoxaemia
 correlated with outcome.\(^6\) 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.\(^6\) 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.\(^6\)
Despite these improvements, the use of the LAL
assay for the rapid diagnosis of gram-negative
septicaemia remains unsatisfactory. It has become
apparent that endotoxin is not always present in
the serum of patients with gram-negative septic ae-
ma and, conversely, that conditions other than
sepsis may result in endotoxaemia.\(^2\) Currently,
the most promising use of the LAL assay in septicaemia
is as a guide to prognosis. Indeed, in a recent study
by Brandtzaeg et al.\(^7\) 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 measure-
ments 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 targeted against mediators of endo-
toxin. 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 endo-
toxin-sensitive species, such as rabbits, and in
animals of lesser sensitivity, such as mice.72,73
Finally, the discovery of naturally occurring anti-
bodies to endotoxin-core structures, in normal
animals as well as in man, led to a series of
retrospective studies in patients with gram-negative
bacteremia, 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 anti-
sperm in high-risk intensive-care patients. Al-
though 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 develop-
ment 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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