Immunotherapy in the management of sepsis

Elizabeth Ann Fagan, Mervyn Singer

Summary

The pathophysiological effects of severe sepsis, septic shock and related syndromes result from tissues damaged by the uncontrolled production of the mediators of inflammation. Early deaths are related primarily to the acute effects of the systemic inflammatory response. Later deaths are related more closely to the consequences of multiple organ dysfunction. Monoclonal antibodies and other immunotherapies have been developed against bacterial products, cytokines and other mediators involved in this systemic inflammatory response. Immunotherapies may improve outcome in the critically ill with sepsis if used early and as part of the therapeutic regimen of antimicrobial agents and intensive care support.

Keywords: immunotherapy, sepsis

Division of Hepatology, Joint
Department of Medicine, University
College of London Medical School and
Royal Free Hospital School of Medicine,
London, UK
EA Fagan

Bloomsbury Institute of Intensive Care
Medicine, University College of London
Medical School, London, UK
M Singer

Correspondence to Dr Mervyn Singer,
Bloomsbury Institute of Intensive Care Medicine,
University College of London Medical School,
Rayne Institute, University Street, London WC1E
6JJ, UK

Accepted 8 August 1994

This paper is based in part on the
Proceedings of the Third Congress on the
Immune Consequences of Trauma, Shock
and Sepsis—mechanisms and therapeutic
approaches, 2–5 March 1994, Munich,
Germany.

Introduction

The morbidity and mortality of patients with severe sepsis and related disorders remain high despite prompt instigation of bactericidal antibiotics and intensive care support. In septic shock, early deaths (< 14 days) are related primarily to the acute effects of the systemic inflammatory response. Later deaths are related more closely to the consequences of refractory hypotension relating to reduced systemic vascular resistance and multiple organ dysfunction.¹ Vital organs are underperfused resulting in multiple organ failure.² In a few cases, myocardial depression contributes to the refractory hypotension.³

The pathophysiological effects of sepsis and related syndromes (see box) result from tissues damaged by the uncontrolled production of the mediators of inflammation.^{4,5} Rapid, effective killing of bacteria is not invariably associated with cessation and reversal of the systemic manifestations of sepsis.

This systemic inflammatory response results from the complex interaction of homeostatic systems with mediators produced by microorganism and host (see boxes) which activates neuroendocrine reflexes, the microvascular endothelium and leucocytes.

Reappraisal of the importance of immune mechanisms may provide a rational approach to the clinical use of immunotherapies designed to interrupt the vicious cycle of events implicated in the high mortality of such patients (see box).

THE SYSTEMIC INFLAMMATORY RESPONSE

Endotoxin (synonym: lipopolysaccharide; LPS) released from lysis of Gram-negative bacteria, exotoxins (enterotoxins) from Gram-positive bacteria, and antigens from a variety of viruses and fungi can trigger a cascade of events leading to the systemic inflammatory response syndrome (SIRS).¹⁰ Similar events can occur with non-infective insults such as burns, trauma, and pancreatitis.

Endotoxaemia has been detected also in some Gram-positive infections and with fungaemia without detectable Gram-negative organisms.^{11,12} This may be related to translocation of endotoxin from the gastrointestinal tract after disruption of the protective mucosa.¹³ This barrier may become compromised following hypoperfusion and reperfusion of the splanchnic circulation, prolonged fasting and, possibly, parenteral nutrition. Bacterial translocation has also been described. The significance of gut translocation in humans is, however, still uncertain.^{14,15}

Endotoxin has three major components. The lipid A moiety is toxic when released and responsible for most of the biological effects. The lipid A component activates mononuclear phagocytes and endothelial cells leading to the release of a variety of cytokines and other mediators of inflammation.¹⁶ An oligosaccharide side-chain, responsible for the O antigenicity, enhances the toxicity of lipid A by increasing the hydrophilic properties of free endotoxin in aqueous media. The O side-chain of bound endotoxin may limit penetration of certain antibiotics into the periplasmic space of the bacterium. This component may also reduce cell lysis by inhibiting binding of complement to the membrane-bound endotoxin.^{17,18} The core polysaccharide is common to all Gram-negative bacteria. This core antigen is hydrophilic and facilitates delivery of lipid A to cellular and protein targets.

INFLAMMATORY MEDIATORS OF SEPTIC SHOCK AND SIRS

The most studied inflammatory response is that associated with endotoxin, either given alone or by injection of Gram-negative bacteria. The resultant activation of monocytes, macrophages and endothelial cells leads to release of a variety of inflammatory mediators (see box).

Tumour necrosis factor (TNF) and interleukin-1 (IL-1) play an important role in the pathogenesis of shock associated with Gram-negative bacterial infection. In man, circulating levels of both cytokines are increased early in septic

Definitions of sepsis and associated disorders (after Bone¹⁰)

Sepsis
Septicaemia associated with a systemic response to infection, including two or more of tachypnoea, hypocapnia, tachycardia, hypothermia, hyperthermia, leucocytosis, leucopenia

Sepsis syndrome (severe sepsis)
Symptoms of sepsis accompanied by failure of one or more organs

Septic shock
Sepsis syndrome and hypotension/hypoperfusion without hypovolaemia (hypotension unresponsive to intravenous fluids and drugs)

Systemic Inflammatory Response Syndrome (SIRS)
Systemic inflammatory response to a variety of severe clinical insults including two or more features associated with sepsis

Systemic inflammatory response

Microorganism mediators, eg:

- endotoxin
- teichoic acid
- peptidoglycans
- muramyl dipeptide
- exotoxins:
 - staphylococcal enterotoxin B toxin-1
 - Pseudomonas exotoxin A

Host mediators, eg:

- cytokines: tumour necrosis factor interleukin-1
- eicosanoids: arachidonic acids prostaglandins
- complement components

Septic shock/septicemia

- 200 000 cases and 100 000 deaths annually in USA (estimated)
- 13th leading cause of death in USA⁶
- 40% cases Gram-negative bacteremia⁷⁻⁹

Some inflammatory mediators of septic shock

Cytokines:

- tumour necrosis factor
- interleukins 1 α , 1 β , 6, 8, and 12
- interferon α and γ
- platelet activating factor

Eicosanoids:

- prostaglandins
- thromboxanes
- leukotriene B₄
- transforming growth factor β ₁

Complement components

shock and following administration of endotoxin.^{19,20} Importantly, the major pathophysiological features of shock can be mimicked by administration of either cytokine in man and animals.^{21,22} Release of TNF from macrophages leads to the production from various tissues of a variety of autocoids, such as platelet-activating factor (PAF), eicosanoids and other cytokines such as IL-1^{23,24} and IL-6.²⁵ These in turn serve to activate other pathways such as the contact system, complement cascade,²⁶ and synthesis of excess nitric oxide. In animal models of sepsis, administration of TNF α alone seems insufficient to produce a shock-like state unless accompanied by PAF.^{27,28} PAF, a phospholipid, amplifies autocatalytically the action of cytokines such as TNF α in shock associated with Gram-negative infection.

THE VASCULAR ENDOTHELIUM
Disruption of the microvascular circulation is a central histopathological feature of septic shock. The autocrine effects of many of the mediators target primarily the vascular endothelium. Synergism between TNF and IL-1 may produce massive damage.²⁹ Expression of endothelial surface glycoproteins, eg, intracellular adhesion molecule 1 (ICAM-1), platelet endothelial-cell-adhesion molecule-1 (PECAM-1), lymphocyte-function-associated-antigen-1 (LFA-1) and the selectins and integrins is increased, leading to enhanced adherence of neutrophils to endothelium.³⁰ PECAM-1 appears to be important for transmigration of neutrophils across the vascular endothelium.

Activation of endothelial cells by endotoxin, TNF and IL-1 leads to a procoagulant state favouring the deposition of fibrin and formation of leukocyte microthrombi. The enzymic cascade responsible for balancing coagulation with fibrinolysis is disrupted. Expression of tissue factor is increased while that of thrombomodulin is reduced. Production of tissue plasminogen activator (tPA) is decreased whereas synthesis of its inhibitor, plasminogen activator inhibitor-1, is augmented.

Arachidonic acid is metabolised to form leukotrienes, thromboxanes and prostaglandins. Vasodilatation results primarily from the action of prostaglandins produced following increased activity of endothelial membrane cyclooxygenase while leukotrienes and thromboxanes induce vasoconstriction. IL-1 and IL-6 activate T cells to produce interferon γ , IL-2, IL-4, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF). These products act to increase permeability of the vascular endothelium as do other agents such as PAF and bradykinin. Endotoxin can activate factor XII (Hageman factor), the protein-initiating activation of the contact system, kallikrein and bradykinin, and complement fragments C3a and C5a. Macrophages are the central source of IL-8, LTB₄ and C5a which are chemo-attractant to polymorphonuclear leucocytes. In turn, these increase further the permeability of the microcirculation and accentuate the inflammatory response by attracting more leucocytes.

Damage to the microvascular circulation is perpetuated by the release of oxygen radicals from disrupted endothelium and various degradative enzymes and aggregation of platelets. Endotoxin and various cytokines, especially IL-1, interferon- γ and TNF α , induce nitric oxide synthase. This enzyme is responsible for the synthesis of nitric oxide (endothelium-derived relaxing factor) in macrophages and smooth muscle cells of the vascular endothelium. Excess production of nitric oxide during sepsis causes vasodilatation and may exacerbate any hypotension. Organ failure and death result primarily from hypoperfusion.

The inflammatory mediators exert complex actions on a variety of other systems. For example, PAF impairs cardiovascular performance and increases pulmonary resistance in animal models.³¹

Immunotherapies in sepsis and SIRS

One rationale is to prevent activation of macrophages and other initiators of the inflammatory cascade by blocking microbial products, such as endotoxin, which can trigger this systemic response. Other alternatives are to block downstream the actions of various mediators released in the systemic inflammatory response or to modulate this response by administration of anti-inflammatory mediators.

Monoclonal antibodies and other immunotherapies (vaccines, polyclonal antibodies) have been developed against many of the initiators and mediators of the systemic inflammatory response (figure). Studies in man are limited but animal studies have led to the development of several leads (see box), some of which are now undergoing phase II and III trials in man.

Some of the leads tested in animal studies

- cytokines
- platelet activating factor
- complement components
- arachidonic acid derivatives
- adhesion molecules

Figure Conceptual model of the systemic inflammatory response following Gram-negative bacteraemia

ANTI-BACTERIAL PRODUCTS

Endotoxin inhibitors
Polyclonal antisera (J5) to a rough mutant of *Escherichia coli* (lacking some of the polysaccharide side-chains) and mouse and human hybrid monoclonal IgM antibodies (HA-1A) have been generated against the lipid A and core components of endotoxin. Selected clinical trials showed an approximate 40% reduction in mortality in patients with sepsis associated with microbiologically proven Gram-negative bacteraemia and septic shock.^{9,32,33}

Lipid X
This monosaccharide precursor of lipid A protected mice and sheep from challenge with lethal doses of endotoxin.^{34,35} Lipid X was found to block competitively the endotoxin priming of neutrophils.³

Bactericidal/permeability increasing protein (BPI)
This product is released by polymorphonuclear leucocytes as part of the defence mechanism against bacterial invasion. BPI binds endotoxin preventing induction of cytokine release. A recombinant product (rBPI23) protected animals from lethal doses of Gram-negative organisms.^{36,37} Multi-centre patient studies are imminent.

Immunoglobulins
Polyclonal and monoclonal immunoglobulins have been produced containing high-titre antibodies to endotoxin³⁸ and high-titre IgG to enhance opsonisation.^{39,40} Preliminary results from three prospective, randomised, (two were also double-blind) controlled clinical trials in severe sepsis and septic shock suggest some benefit (reduced mortality) when compared with placebo or no supplemental treatment. One further study showed benefit for polyclonal IgM but not monoclonal (IgG or IgM) preparations.⁴¹

Clinical limitations of anti-endotoxin therapies

The efficacy of some of these products remains uncertain in clinical practice. Also, concern remains over the validity of extrapolating data from results of animal studies. The design and analyses of the trials with J5 and HA-1A have been criticised. Survival was not improved in patients without microbiologically proved bacteraemias who received HA-1A when compared to placebo.⁹ HA-1A has been withdrawn from general clinical use due to fears about its potential toxicity in patients without proven Gram-negative sepsis. Also, the mode of action of HA-1A remains unclear despite early claims that it binds specifically to endotoxin.

Gram-negative bacteria vary significantly in the amount of endotoxins they produce.⁴² Results from studies *in vitro* suggest that release of endotoxin and, subsequently, cytokines such as TNF relates in part to the rate of killing of bacteria and its release from lysed cell walls.^{43,44} In septic shock, levels of free endotoxin do not correlate invariably with measures of disease severity, such as changes in cardiovascular response, laboratory indices and clinical outcome.¹² Assays to measure levels of endotoxin have improved but problems remain with control of sensitivity, specificity and inter-assay variation. The significance of endotoxin remaining bound to bacteria cell walls is unclear.

Inhibitors of the host response

ANTI-CYTOKINES

Administration of monoclonal antibodies to TNF in rabbits with meningitis prevented the development of shock induced by endotoxin and *Escherichia coli* and improved survival.^{21,45,46} Anti-TNF antibodies also protected neutropenic rats from lethal infection with *Pseudomonas aeruginosa*.⁴⁷ This protective effect was also shown in non-human primates given anti-TNF α after challenge with endotoxin.⁴⁸

The mouse anti-human monoclonal antibody CB6 has been tested in the US in a multinational clinical trial (Phase II; open label, dose-escalation) of severe sepsis.⁴⁹ The small sample size in each group (20 patients) precluded any statistical analysis. Although there was no overt toxicity, many patients had detectable anti-murine antibodies as well as immune complexes to CB6. In contrast, preliminary results using different murine monoclonal anti-TNF α preparations from other multicentre, double-blind controlled trials in the US⁵⁰ and the UK⁵¹ showed these to be safe and associated with reduced early mortality in shocked, though not in non-shocked, patients.

The actions of TNF α and IL-1 (α and β forms) are modulated primarily at the level of their receptors. An antagonist to TNF α has been discovered.⁵² There is concern over the feasibility of using inhibitors of TNF as anti-inflammatory agents. Administration of soluble receptors to TNF (sTNFR) resulted in excess mortality, possibly due to the release of bound TNF from the construct.⁵¹

An antagonist to the IL-1 receptor (IL-1ra) has been cloned. This recombinant product, rhIL-1ra, is nonglycosylated and blocks competitively the action of IL-1 without any detectable agonist activity.⁵³ IL-1ra reduced the mortality in animal models of shock associated with endotoxaemia.⁵⁴ Permanent adherence of leukocytes to the vascular endothelium in rat liver was reduced following administration of IL-1ra following haemorrhagic shock.⁵⁵

A randomised, double-blind controlled trial of rhIL-1ra involving 63 centres in Europe and the US was carried out in patients with severe sepsis and septic shock, stratified for organ dysfunction, including adult respiratory distress syndrome, disseminated intravascular coagulation and renal and hepatic dysfunction (table).⁵⁶ Survival time was dose-dependent and increased significantly for IL-1ra compared with placebo in all groups (excluding hepatic). Although overall survival was not significantly different between IL-1ra and placebo

Table Recombinant human interleukin-1 receptor antagonist (rhIL-1ra): 28-day percentage mortality in sepsis-induced organ dysfunction (after Fisher *et al*⁵⁶)

	Number	Placebo	rhIL-1ra* (1 mg/kg/h)	rhIL-1ra* (2 mg/kg/h)	p-value
ARDS	233	49	43	34	0.04
DIC	124	64	58	38	0.03
Renal failure	267	49	41	33	0.01
Liver failure	235	43	44	35	0.10

ARDS: adult respiratory distress syndrome; DIC: disseminated intravascular coagulation.
*72 h continuous infusion.

groups, *post-hoc* analysis did show a statistically significant benefit in those patients with an APACHE III risk of death exceeding 24%.⁵⁷ This finding has formed the basis of another large prospective multi-centre study which is currently in progress.

ANTI-INFLAMMATORY CYTOKINES

IL-4, IL-10, IL-13 and TGF β inhibit the production of pro-inflammatory cytokines such as IL-1, IL-6, TNF α and chemokines MIP $_{1\alpha}$ and IL-8 following activation of monocytes by endotoxin.

IL-10, a cytokine produced by activated T cells, B cells, monocytes and macrophages, occurs naturally and may have potential as an anti-inflammatory agent. Administration of IL-10 *in vitro* inhibits the production of pro-inflammatory cytokines, especially IL-1, IL-6, TNF α and chemo-attractants (IL-8 among others). IL-10 also enhances the production of IL-1ra and down-regulates expression of MHC class II antigens. IL-10 production by T cells can be inhibited by anti-IL-10 monoclonal antibodies and inhibitors of eicosanoids. Preliminary data showed that administration of IL-10 prevented death in mice following endotoxin-induced toxic shock.⁵⁸

IL-13 has also been cloned; its gene is linked to that for IL-4 on chromosome 5.^{59,60} Many of its biological properties are similar to IL-4, including enhancing expression of MHC class II antigens on monocytes and CD23.

PLATELET ACTIVATING FACTOR (PAF)

Studies are in progress in non-human primates and phase II and III trials in man using more than 15 different preparations, including recombinant and naturally occurring products which act as receptor antagonists to PAF.^{31,61,62} Randomised, double-blind controlled trials in severe sepsis showed these products to be safe. Mortality was reduced significantly compared with placebo in microbiologically proved Gram-negative infection for BN 52021, a naturally occurring PAF antagonist although, surprisingly, no beneficial effect was seen with infections of non-Gram-negative origin.⁶²

INHIBITORS OF COMPLEMENT COMPONENTS

Monoclonal antibodies directed against factor XII protected against hypotension, but not disseminated intravascular coagulation, in primate models of sepsis.^{63,64} C1 esterase inhibitor (C1-INH) is the main inhibitor of the classical pathway for activation of complement. Administration of C1-INH in human sepsis reduced the degree of hypotension and capillary leakage associated with IL-2.⁶⁵

ARACHIDONIC ACID DERIVATIVES

Thromboxane A₂, prostaglandins and leukotrienes exert important effects on the microvascular circulation and platelets in sepsis and septic shock. Studies are underway to assess the clinical efficacy of monoclonal antibodies as inhibitors but concern remains over their specificity. Monoclonal antibodies against phospholipase A₂, an enzyme involved in the release of arachidonic acid, also inhibit synthesis of prostaglandins and leukotrienes.⁶⁶

ANTI-ADHESION MOLECULES

Enhanced expression of adhesion molecules is essential to the normal response of the host to many diseases, including infections. Anti-adhesion molecules could act to limit the damage to host tissues caused by the inflammatory response. Studies are in progress on a variety of causes of multiple organ dysfunction, including ischaemia/reperfusion injury of grafts and in the adult respiratory distress syndrome. Many strategies are under consideration including blocking their ligands by antibodies, soluble receptors, peptide analogues, oligosaccharides (anti-lectins) and anti-sense nucleotides.⁶⁷ Monoclonal antibodies directed against an alpha-chain (anti-CD11a, b, or c) of the glycoprotein of the integrins prevents binding of polymorphonuclear leukocytes to their ligand (ICAM-1). In various animal studies, administration of monoclonal antibodies to CD11b and ICAM-1 reduced the tissue injury caused by endotoxaemia, activation of complement, and ischaemia-reperfusion.^{68,69}

BRADYKININ ANTAGONISTS

Different bradykinin receptors have been recognised. The BK₂ receptor is constitutive while the BK₁ receptor is also inducible and upregulated in inflammatory conditions such as sepsis. The bradykinin antagonist CP-0127 significantly increased survival in animals models of endotoxic shock⁷⁰ and a multi-centre Phase III human sepsis study using this agent is currently being conducted in the US.

Rationale of immunotherapy for sepsis
<ul style="list-style-type: none">• block triggers of inflammatory response such as endotoxin• block pro-inflammatory mediators such as TNF, PAF• enhance anti-inflammatory mediators such as IL-1 receptor antagonist

Difficulties in conducting human sepsis immunotherapy studies
<ul style="list-style-type: none">• heterogeneous patient populations (underlying disease process and co-morbidities)• relatively low yield of positive microbiological diagnosis• difficulty in rapid confirmation of appropriateness of therapy, eg, presence of Gram-negative bacteraemia, endotoxaemia, high TNF levels• timing and duration of treatment relative to onset of sepsis• non-standardisation of other treatments, eg, vasoactive drugs, antibiotics• monotherapy unlikely to be a panacea• expense

Clinical scope and limitations

All observations point to the need to administer immunotherapies early in sepsis. In clinical practice, strategies targeting mediators of SIRS are more realistic than those focusing only on inhibiting initiators of this cascade, such as endotoxin. However, a prophylactic role in high-risk patients may well suit these agents.

Development of sepsis and shock is independent of the continuous release of endotoxin and other mediators. Minute quantities of endotoxin and mediators seem sufficient to account for the systemic effects. Also, endotoxaemia may outlive detectable Gram-negative bacteraemia.¹² Consequently, inhibition of release would have to be complete to ensure clinical efficacy. The release and relative contribution of the different mediators vary between patients and target organs. Damage to the endothelium and critical organs poses a new threat – a second wave effect – from increased release of mediators.

A major potential benefit of targeting cytokines such as TNF and IL-1 is that such treatments can be directed at Gram-positive as well as Gram-negative infections. The alternative hypothesis is that these cytokines are part of the normal defence mechanism; administration of their antagonists may be harmful.^{16,71} TNF and IL-1 mediate the induction of nitric oxide synthase by endotoxin. Although nitric oxide in excess may exacerbate hypoperfusion of vital organs and tissues, inhibition of nitric oxide synthase may prove equally deleterious by impairing further the perfusion of critical organs and removing the cytotoxic properties of nitric oxide.⁷²

MULTIPLE THERAPIES

Any monotherapy is unlikely to be universally effective in septic shock. Only around one third of patients with septic shock have detectable levels of TNF, possibly due to its short half-life and release in bursts. Elevated levels of TNF alone do not account for the development of shock and are not exclusive to sepsis. Comparable levels are detectable in a variety of chronic conditions, including autoimmune disease. Also, in murine models of lethal infection, anti-TNF antibodies protected against Gram-negative shock associated with *Escherichia coli* but not *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*.^{73,74}

Assessment of the clinical efficacy of combination therapies throws into focus the difficulties in studying the critically ill. Dissection of the relative importance of any therapy, especially in combination with others, will require large numbers of patients stratified for disease, severity of illness and a multitude of biochemical and pharmacological variables.

Immunotherapies must join a variety of pharmacological inhibitors being tested in animal models.¹⁰ Drugs which inhibit eicosanoids (prostaglandins), alter calcium fluxes or prevent damage by oxygen-free radicals (antioxidants), have shown benefit in *in vitro* studies of cell death and animal models of septic shock.

Measurement of the mediators involved in the SIRS remains unreliable; reliance on blood levels to assess paracrine (local) effects is unlikely to reflect faithfully events occurring in sepsis. The clinical significance of the development of antibodies and immune complexes to certain murine monoclonal products, such as anti-CB6 (anti-TNF α) is uncertain.⁴⁷

Also, production and regulation of the mediators, especially the cytokines, are influenced by extraneous factors such as drugs (chlorpromazine, *N*-acetyl cysteine, pentoxifylline, naloxone and anaesthetic agents among many others), corticosteroids and neuropeptides (α -melanocyte-stimulating hormone [α -MSH], macrophage inhibitory factor [MIF]) of the neuroendocrine axis.

Results from animal studies must be extrapolated with caution. Administration of bolus doses of endotoxin bears little resemblance to events occurring in sepsis. Also, most cytokines are species-specific. In a mouse model of sepsis, anti-endotoxin antibodies protected against the lethal effects of challenge with endotoxin without measurable effects on the rise in levels of TNF α .⁷⁴ However, TNF may play a role in defence of the host against invasion by some microorganisms.⁷¹

Defences of bacteria

Bacteria possess several properties which favour invasion and spread. These defences should be addressed in any antimicrobial strategy. Production of specific adhesins on surface pili favour adherence to mucosal surfaces. The O side-chain of bound endotoxin may limit penetration of certain antibiotics into the periplasmic space of the bacterium and also reduce cell lysis by inhibiting binding of complement to the membrane-bound endotoxin^{17,18} in the absence of antibody. Surface encapsulation confers some protection against phagocytosis by neutrophils. Certain bacteria, such as *P aeruginosa*, produce enzymes

(elastases) and toxins (exotoxin A) which favour necrosis by inhibiting protein synthesis of tissue.

Certain antibiotics of the β -lactam class, particularly some cephalosporins and aztreonam cause release of endotoxin following binding to the cell wall of the microorganism.^{75,76} Their ability to induce *in vitro* release of endotoxin and, subsequently, TNF α is independent of the degree of lysis of the bacterial cell wall but related to the dose of antibiotic. In a rat sepsis model, treatment with imipenem or ceftazidime resulted in only a transient rise in IL-6 while aztreonam produced much higher levels and a significant increase in mortality.⁷⁷ Another study in septic mice showed significantly greater *in vivo* chemotherapeutic protection with imipenem compared to ceftazidime.⁷⁸ The clinical significance of these observations remains uncertain.

- 1 Knaus WA, Harrell FE, Fisher CJ Jr, *et al.* The clinical evaluation of new drugs for sepsis. A prospective study design based on survival analysis. *JAMA* 1993; 270: 1233–41.
- 2 Parker MM, Shelhamer JH, Natanson C, Alling DW, Parillo JE. Serial cardiovascular variables in survivors and nonsurvivors of human septic shock: heart rate as an early predictor of prognosis. *Crit Care Med* 1987; 15: 923–9.
- 3 Parillo JE. Septic shock in humans. *Ann Intern Med* 1990; 113: 227–42.
- 4 Border JR. Hypothesis: multiple systems failure and the macrophage. *Arch Surg* 1988; 123: 285–6.
- 5 Michie HR, Wilmore DW. Sepsis, signals and the surgical sequelae (an hypothesis). *Arch Surg* 1990; 125: 17–22.
- 6 US Centers for Disease Control. Increase in national hospital discharge rates for septicemia—United States, 1979–1987. *MMWR* 1987; 39: 31–4.
- 7 Kreger BE, Craven DE, McCabe WR. Gram-negative bacteremia IV. Re-evaluation of clinical features and treatment in 612 patients. *Am J Med* 1980; 68: 344–55.
- 8 Tran DD, Groeneveld AB, van der Meulen J, Nauta JJ, Strack van Schijndel RJ, Thijs LG. Age, chronic disease, sepsis, organ failure, and mortality in a medical intensive care unit. *Crit Care Med* 1990; 18: 474–9.
- 9 Ziegler EJ, Fisher CJ Jr, Sprung CL, *et al.* Treatment of gram negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin – a randomized, double-blind, placebo-controlled trial. *N Engl J Med* 1991; 324: 429–36.
- 10 Bone RC. A critical evaluation of new agents for the treatment of sepsis. *JAMA* 1991; 266: 1686–91.
- 11 Van Deventer SJH, Buller HR, ten Cate JW, Sturk A, Pauw W. Endotoxemia: an early predictor of septicaemia in febrile patients. *Lancet* 1988; i: 605–9.
- 12 Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, Parillo JE. Endotoxemia in septic shock. *Chest* 1991; 99: 169–75.
- 13 Raven HA, Rowley D, Jenkins C, Fine J. On the absorption of bacterial endotoxin from the gastrointestinal tract of the normal and shocked animal. *J Exp Med* 1960; 112: 783–92.
- 14 Roumen RM, Frieling JT, van Tits HW, van der Vliet JA, Goris RJ. Endotoxemia after major vascular operations. *J Vasc Surg* 1993; 18: 853–7.
- 15 Munster AM, Smith-Meek M, Dickerson C, Winchurch RA. Translocation. Incidental phenomenon or true pathology? *Ann Surg* 1993; 218: 321–6.
- 16 Bone RC. The pathogenesis of sepsis. *Ann Intern Med* 1991; 115: 457–69.
- 17 Ciurana C, Thomas JM. Role of lipopolysaccharide and complement in susceptibility of *Klebsiella pneumoniae* to nonimmune serum. *Infect Immun* 1987; 55: 2741–6.
- 18 Portat R, Johns MA, McCabe WR. Selective pressures and lipopolysaccharide subunits as determinants of resistance of clinical isolates. *Infect Immun* 1987; 55: 320–8.
- 19 Tracey KJ, Beutler B, Lowry SF, *et al.* Shock and tissue injury induced by recombinant human cachectin. *Science* 1986; 234: 470–4.
- 20 Cannon JG, Tompkins RG, Gelfand JA, *et al.* Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 1990; 161: 79–84.
- 21 Hesse DG, Tracey KJ, Fong Y, *et al.* Cytokine appearance in human endotoxemia and primate bacteremia. *Surg Gynecol Obstet* 1988; 166: 147–53.
- 22 Natanson C, Eichenholz PW, Danner RL, *et al.* Endotoxin and tumor necrosis factor challenges in dogs simulates the cardiovascular profile of human septic shock. *J Exp Med* 1989; 169: 823–32.
- 23 Sun XM, Hsueh W. Bowel necrosis induced by tumor necrosis factor in rats mediated by platelet-activating factor. *J Clin Invest* 1988; 81: 1328–31.
- 24 Wang J, Dunn MJ. Platelet-activating factor mediates endotoxin-induced renal insufficiency in man. *Am J Physiol* 1987; 253: F1283–9.
- 25 Petrak RA, Balk RA, Bone RC. Prostaglandins, cyclo-oxygenase inhibitors, and thromboxane synthesis inhibitors in the pathogenesis of multiple organ failure. *Crit Care Clin* 1989; 5: 303–14.
- 26 van der Poll T, Butler HR, ten Cate H, *et al.* Activation of coagulation after administration of tumor necrosis factor to normal subjects. *N Engl J Med* 1990; 322: 1622–7.
- 27 Myers AK, Robey JW, Price WM. Relationships between tumour necrosis factor, eicosanoids and platelet-activating factor as mediators of endotoxin-induced shock in mice. *Br J Pharmacol* 1990; 99: 499–502.
- 28 Sun XM, Hsueh W, Torre-Amione G. Effects of 'in vivo' priming on endotoxin-induced hypotension and tissue injury. The role of PAF and tumor necrosis factor. *Am J Pathol* 1990; 136: 949–56.
- 29 Okusawa S, Gelfand JA, Ikejima T, *et al.* Interleukin 1 induces a shock-like state in rabbits: synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. *J Clin Invest* 1988; 81: 1162–72.
- 30 Cotran RS, Pober JS. Cytokine-endothelial interactions in inflammation, immunity, and vascular injury. *J Am Soc Nephrol* 1990; 1: 225–35.
- 31 Sanchez Crespo M. Potential of PAF receptor antagonists in the treatment of septic shock. *DN & P* 1993; 6: 78–87.
- 32 Ziegler EJ, McCutchan JA, Fierer J, *et al.* Treatment of Gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med* 1982; 307: 1225–30.
- 33 Baumgartner J-D, Glauser MP, McCutchan JA, *et al.* Prevention of Gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. *Lancet* 1985; ii: 59–63.
- 34 Proctor RA, Will JA, Burhop KE, Raetz CR. Protection of mice against lethal endotoxemia by a lipid A precursor. *Infect Immun* 1986; 52: 905–7.
- 35 Golenbock DT, Will JA, Raetz CR, Proctor RA. Lipid X ameliorates pulmonary hypertension and protects sheep from death due to endotoxin. *Infect Immun* 1987; 55: 2471–6.
- 36 Kohn FR, Ammons WS, Lin Y, Kung AHC. Protective effect of an NH₂-terminal fragment of bactericidal/permeability increasing protein in animal models of lipopolysaccharide or bacterial challenge. *Intensive Care Med* 1994; 20 (suppl 1): S33 (abstract).
- 37 Yao YM, Bahrami S, Leichtfried G, Redl H, Schlag G. Effects of bactericidal/permeability increasing protein (BPI) against haemorrhage-induced endotoxemia and mortality in rats. *Intensive Care Med* 1994; 20 (suppl 1): S479 (abstract).
- 38 Schedel I, Dreikhausen U, Nentwig B, *et al.* Treatment of gram-negative septic shock with an immunoglobulin preparation. *Intensive Care Med* 1994; 20 (suppl 1): S591 (abstract).
- 39 Dominioni L, Gennari R, Dionigi R. Immunotherapy of sepsis with Sandoglobulin. *Intensive Care Med* 1994; 20 (suppl 1): S592 (abstract).
- 40 Werdan K and the SBITS Study Group. Polyglobin N⁸. *Intensive Care Med* 1994; 20 (suppl 1): S593 (abstract).
- 41 Beger D, Torab F, Bolke E, Beger HG. Endotoxin neutralizing capacity of polyclonal human IgM. *Intensive Care Med* 1994; 20 (suppl 1): S602 (abstract).

- 42 Danner RL, Natanson C, Elin RJ, *et al.* *P. aeruginosa* compared to *E. coli* produces less endotoxemia but more cardiovascular dysfunction and mortality in a canine model of septic shock. *Chest* 1990; 98: 1480-7.
- 43 Shenep JL, Barton RP, Morgan KA. Role of antibiotic class in the rate of liberation of endotoxin during therapy for experimental Gram-negative bacterial sepsis. *J Infect Dis* 1985; 151: 1012-8.
- 44 Eng RHK, Smith SM, Fan-Havard P, Ogbara T. Effect of antibiotics on endotoxin release from Gram-negative bacteria. *Diagn Microbiol Infect Dis* 1993; 16: 185-9.
- 45 Tracey KJ, Fong Y, Hesse DG, *et al.* Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. *Nature* 1987; 330: 662-4.
- 46 Tauber MG, Shibl AM, Hackbarth CJ, Larrick JW, Sande MA. Antibiotic therapy, endotoxin concentration in cerebrospinal fluid, and brain edema in experimental *Escherichia coli* meningitis in rabbits. *J Infect Dis* 1987; 156: 456-62.
- 47 Opal SM, Cross AS, Kelly NM, *et al.* Efficacy of monoclonal antibody directed against tumor necrosis factor in protecting neutropenic rats from lethal infection with *Pseudomonas aeruginosa*. *J Infect Dis* 1990; 161: 1148-52.
- 48 Hinshaw LB, Tekamp-Olsen P, Chang AC, *et al.* Survival of primates in LD100 septic shock following therapy with antibody to tumor necrosis factor (TNF alpha). *Circ Shock* 1990; 30: 279-92.
- 49 Opal SM, Fisher CJ, Dhainaut JF, Bodmer M. Anti-tumor necrosis factor strategies - the CB6 monoclonal antibody study. *Intensive Care Med* 1994; 20 (suppl 1): S594 (abstract).
- 50 Wherry J, Abraham E, Wunderink R, *et al.* Efficacy and safety of murine monoclonal antibody to human tumor necrosis factor (TNF α MAbs). *Intensive Care Med* 1994; 20 (suppl 1): S595 (abstract).
- 51 Cohen J. Tumor necrosis factor as a therapeutic agent. *Intensive Care Med* 1994; 20 (suppl 1): S29 (abstract).
- 52 Seckinger P, Isaaz S, Dayer JM. Purification and biologic characterization of a specific tumor necrosis factor alpha inhibitor. *J Biol Chem* 1989; 264: 1966-73.
- 53 Hannum CH, Wilcox CJ, Arend WP, *et al.* Interleukin-1 receptor antagonist activity of human interleukin-1 inhibitor. *Nature* 1990; 343: 336-40.
- 54 Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* 1990; 348: 550-2.
- 55 Bauer C, Marzi I, Fellger H, Bauer M, Larsen R. Interleukin-1 receptor antagonist reduces leukocyte-endothelial interactions in the liver following hemorrhagic shock. *Intensive Care Med* 1994; 20 (suppl 1): S160 (abstract).
- 56 Fisher CJ, Pribble JP, Opal SM, *et al.* Recombinant human interleukin-1 receptor antagonist (rhIL-1ra) increases survival time in patients with sepsis-induced organ dysfunction. *Intensive Care Med* 1994; 20 (suppl 1): S598 (abstract).
- 57 Knaus WA and the rhIL-1ra Phase III Sepsis Study Group. The relationship between patient risk and efficacy of immunotherapy of sepsis. *Intensive Care Med* 1994; 20 (suppl 1): S600 (abstract).
- 58 de Vries JE. Anti-inflammatory properties of IL-10. *Intensive Care Med* 1994; 20 (suppl 1): S209 (abstract).
- 59 Minty A, Chalon P, Derocq, JM, *et al.* Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature* 1993; 362: 248-50.
- 60 McKenzie AN, Largaespada DA, Sato A, *et al.* Structural comparison and chromosomal localization of the human and mouse IL-13 genes. *J Immunol* 1993; 150: 5436-44.
- 61 Thompson W, Moldawer LL, Lowry SF. The metabolic effects of platelet-activating factor antagonism in lethal primate bacteremia and human endotoxemia. *Intensive Care Med* 1994; 20 (suppl 1): S587 (abstract).
- 62 Dhainaut JF, Tenaillon A, Guinot Ph and the Sepsis Study Group. PAF antagonist (BN 52021) in severe Gram-negative sepsis. *Intensive Care Med* 1994; 20 (suppl 1): S600 (abstract).
- 63 Colman RW. The role of the contact system in systemic inflammatory response syndrome. *Intensive Care Med* 1994; 20 (suppl 1): S1 (abstract).
- 64 Jansen PM, Pixley RA, Chang ACK, *et al.* Inhibition of factor XII in septic baboons attenuates activation of complement and fibrinolytic systems and reduces the release of neutrophil elastase. *Intensive Care Med* 1994; 20 (suppl 1): S6 (abstract).
- 65 Hack CE, Ogilvie AC, Wagstaff J, Thijs LG. C1-inhibitor substitution therapy in the vascular leak syndrome induced by high doses of interleukin-2. *Intensive Care Med* 1994; 20 (suppl 1): S7 (abstract).
- 66 Bone RC. Phospholipids and their inhibitors: a critical evaluation of their role in the treatment of sepsis. *Crit Care Med* 1992; 20: 884-90.
- 67 Doerschuk CM. Anti-adhesion molecule strategies as a therapeutic modality: an overview. *Intensive Care Med* 1994; 20 (suppl 1): S104 (abstract).
- 68 Wortel CH. Modulation of neutrophil-endothelial cell adhesion with anti-CD11/CD128 monoclonal antibodies as a therapeutic modality. *Intensive Care Med* 1994; 20 (suppl 1): S106 (abstract).
- 69 Menger MD, Vollmar B. Anti-ICAM-1 monoclonal antibody in hepatic ischemic injury. *Intensive Care Med* 1994; 20 (suppl 1): S107 (abstract).
- 70 Whalley ET, Solomon JA, Modafferi DM, Bonham KA, Cheronis JC. CP-0127, a novel potent bradykinin antagonist, increases survival in rat and rabbit models of endotoxin shock. *Agents Actions Suppl* 1992; 38 (Pt 3): 413-20.
- 71 Smith JG, Magee DM, Williams DM, Graybill JR. Tumor necrosis factor-alpha plays a role in host defense against *Histoplasma capsulatum*. *J Infect Dis* 1990; 162: 1349-53.
- 72 Cobb JP, Cunnion RE, Danner RL. Nitric oxide as a target for therapy in septic shock. *Crit Care Med* 1993; 21: 1261-3.
- 73 Silva AT, Bayston KF, Cohen J. Prophylactic and therapeutic effects of a monoclonal antibody to tumor necrosis factor-alpha in experimental Gram negative shock. *J Infect Dis* 1990; 162: 421-7.
- 74 Silva AT, Appelmeik BJ, Buurman WA, Bayston KF, Cohen J. Monoclonal antibody to endotoxin core protects mice from *Escherichia coli* sepsis by a mechanism independent of tumor necrosis factor and interleukin-6. *J Infect Dis* 1990; 162: 454-9.
- 75 Simon DM, Koenig G, Trenholme GM. Differences in release of tumor necrosis factor from THP-1 cells stimulated by filtrates of antibiotic-killed *Escherichia coli*. *J Infect Dis* 1991; 164: 800-2.
- 76 Arditi M, Kabat W, Yogev R. Antibiotic-induced bacterial killing stimulates tumor necrosis factor-alpha release in whole blood. *J Infect Dis* 1993; 167: 240-4.
- 77 Dofferhorf ASM. Release of endotoxin and cytokines during the antibiotic treatment of gram negative infection. *Intensive Care Med* 1994; 20 (Suppl 1): S19 (abstract).
- 78 Morrison DC, Bucklin S, Leeson M, Fujiwara Y. Bacteremia vs endotoxemia in antibiotic treatment of experimental Gram negative sepsis. *Intensive Care Med* 1994; 20 (suppl 1): S21 (abstract).