Emerging Therapies for Sepsis and Septic Shock

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Abstract

Despite advances in antimicrobial therapy and medical support, septic shock remains a leading cause of death. Emerging adjunctive therapy for septic shock can be divided into those directed against bacterial components, those directed against host-derived inflammatory-mediators and those designed to limit tissue damage. All trials of new adjunctive therapies for sepsis and septic shock conducted to date have failed to show efficacy. Therapies against endotoxin, tumour necrosis factor, interleukin-1 and platelet activating factor did not reduce mortality. Future effective therapies will probably use combination of agents depending upon the nature of the infection and the type of patient.

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Introduction

Septic shock remains a major cause of morbidity and mortality among hospitalised patients despite advances in antimicrobial therapy and medical support.[1]

Septic shock has traditionally been recognised as a consequence of gram-negative bacterial infection but may also be caused by gram-positive organisms and fungi.

Recently, the somewhat vague term sepsis has been replaced by terms for three clinical syndromes defining a progressive increase in the systemic inflammatory response to infection: sepsis, severe sepsis and septic shock (Table I).[2] Emerging therapies for sepsis and septic shock, based on modulation of immune responses, have been aimed at trying to improve survival of patients.

TABLE I: CONSENSUS CONFERENCE DEFINITIONS FOR SEPSIS AND RELATED CONDITION

Systemic Inflammatory Response Syndrome (SIRS)	The systemic inflammatory response to a variety of severe clinical insults —manifest by 2 or more of the following: (1) Temperature >38°C or <36°C (2) Heart rate >90 beats/min (3) Respiratory rate >20 breaths/min or P_aCO_2 <32 mmHg (4) White blood cell count >12 000/mm^3 or <4000/mm^3 or >10% immature (band) forms
Sepsis	The systemic response to infection i.e. SIRS plus a culture-documented infection.
Severe Sepsis	Sepsis associated with organ dysfunction, hypoperfusion or hypoperfusion including but not limited to lactic acidosis, oliguria or acute alteration in mental status.
Septic Shock	Hypotension (despite adequate fluid resuscitation) plus hypoperfusion abnormalities. Hypotension is defined as a systolic BP <90 mmHg or a reduction of >40 mmHg from baseline in the absence of other causes of hypotension.
Multiple Organ Dysfunction Sundrome (MODS)	Presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.

Organ Failure in Sepsis

As sepsis advances toward shock, there is an increased sympathetic tone resulting in tachycardia associated with hypotension. This is followed by an increase in respiratory drive manifested by tachypnoea and hypernoea. Hypoperfusion of the liver and periphery leads to lactic acidosis. Hypoperfusion of the central nervous system leads to stupor and coma. Renal manifestations include azotemia and oliguria that result from renal tubular injury.[3]

The characteristic haemodynamic profile seen in patients with septic shock is a hyperdynamic pattern with an increased cardiac output as systemic vascular resistance falls. Although myocardial depression is common in shock, a decreased cardiac output is unusual.[4] Animal studies have shown that tumour necrosis factor (TNF)

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injected into guinea pigs causes myocardial depression and decreased responsiveness to norepinephrine.[5] There is an increasing body of evidence that nitric oxide (endothelium-derived relaxing factor) mediates the myocardial depression produced by the cytokines that is seen in sepsis.[6]

As vasomotor tone falls and capillaries leak in sepsis, there is a great need to replace intravascular volume. Underestimating the amount of fluids is a serious error in the management of septic shock. If fluid replacement alone fails to correct blood pressure despite a normal or elevated cardiac output, administration of a vasopressor such as dopamine or norepinephrine is indicated. Failure of sympathomimetic agents in the presence of an adequate preload may be associated with acidosis (pH <7.3), hypocalcaemia, adrenal insufficiency or hypoglycaemia.[7]

Pathogenesis of Septic Shock

The prime initiator of gram-negative bacterial septic shock is endotoxin, a lipopolysaccharide(LPS)-phospholipid-protein complex present in the bacterial outer membrane. LPS is made largely of a structurally and antigenically diverse oligosaccharides (O antigen), the core and lipid A. Lipid A is a highly conserved disaccharide with polar phosphates responsible for most of the toxicity of endotoxin.

In most species, the injection of LPS is associated with a rapid onset of fever, hypotension and neutropenia.[8] The adverse effects observed with endotoxin result from its capacity to cause the release of various endogenous mediators and to activate the coagulation and complement cascade (Fig. 1).

Cytokines are an important group of mediators produced by macrophages, lymphocytes and endothelial cells. These include pro-inflammatory cytokines such as tumour necrosis factor (TNFα), interleukins (IL-1, IL-6, IL-8) and interferon (IFNγ) and counter-regulatory or anti-inflammatory mediators such as IL-4, IL-10, IL-13, transforming growth factor (TGF) B, IL-1 receptor antagonist (IL-1ra) and soluble TNF receptors.[9,10]

Currently, two biochemical elements are known to recognise LPS: a serum protein, LPS-binding protein (LPB) which is an acute-phase reactant and another protein, CD_{14} that is either soluble or on the surface of macrophages and neutrophils (Fig. 2).[11] CD_{14} mediates signalling to the nuclei of responding cells by an unknown mechanism.

Besides endotoxin from gram-negative bacteria, exotoxins, enterotoxins and constituents of the cell wall of gram-positive bacteria also cause septic shock. Enterotoxins and exotoxins released from gram-positive bacteria may initiate the inflammatory cascade through their direct effects on macrophages and other cells and

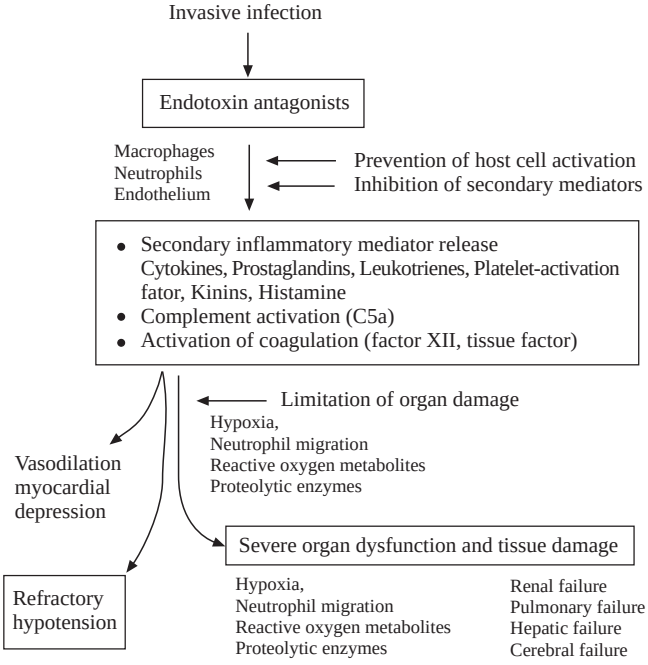

Fig. 1. Pathogenesis of septic shock—potential sites of therapy for septic shock.

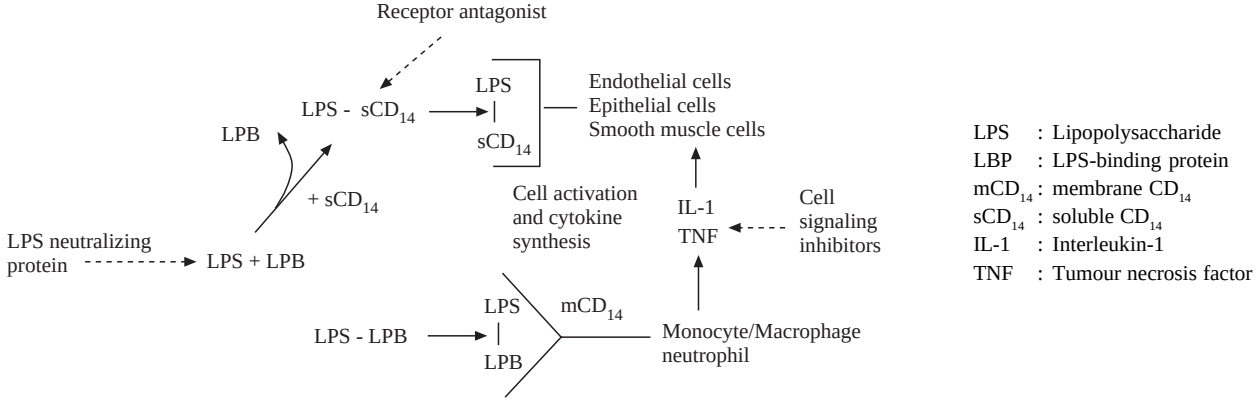

Fig. 2. Cellular responses to lipopolysacccharide (endotoxin) and potential sites for inhibition.

by their ability to act as superantigens.[12] Staphylococcal enterotoxins, toxic shock syndrome toxin 1 (TSST-1), prompts human monocytes to release IL-1 (even more than endotoxin) and TNF.[13]

Therapeutic Approaches to Septic Shock

While therapy for underlying infection has emphasized appropriate antibiotics and drainage of abscesses, therapy for specific manifestations of sepsis and septic shock is also necessary. This includes critical care monitoring, aggressive fluid resuscitation and if shock continues, use of inotropes and vasopressors, ventilatory support to increase oxygen delivery and nutritional support.

Approaches to emerging adjunctive therapy for septic shock fall into three main categories:

(1) Strategies Directed Against Bacterial Components

Therapy that target the initial interaction of bacterial products (notably gram-negative bacterial endotoxin) with inflammatory cells is likely to be of most benefit when administered early in sepsis before widespread vascular injury occur or prophylactically to high-risk patients. One drawback to such an approach is that they are specific to a single class of organisms e.g. anti-endotoxin therapy for gram-negative bacterial infections.

a) Antibodies to endotoxin

Two monoclonal antibodies against endotoxin have been the subject of large scale clinical trials and some controversy.[13-16] The human monoclonal antibody, HA-1A, was initially reported to have no overall benefit in 543 patients with sepsis when compared with human serum albumin placebo. However, HA-1A appeared to afford significant protection to a subgroup of 200 patients with gram-negative bacteraemia.[13] The second placebo-controlled study with HA-1A documented a lack of overall clinical benefit of HA-1A and a non-significant survival disadvantage among patients without gram-negative bacteraemia.[14]

The murine monoclonal antibody, E5, initially was reported to reduce mortality and enhance the resolution of organ failure in 137 patients with gram-negative sepsis without shock compared with 179 patients with shock.[15] A second multicentre randomised controlled trial did not confirm the benefit of this agent in reducing mortality.[16]

Until further data become available, neither HA-1A nor E5 can be recommended for the treatment of gram-negative sepsis. The important potential problem with the clinical use of anti-endotoxin agents is getting such drugs to the patients early enough before the proinflammatory cytokine expression has occurred.

b) LPS-receptor antagonist

Potential sites of intervention in LPS-induced cellular activation are depicted in Figure 2. Inhibition of LPS / LPB binding to cells by monoclonal antibodies to CD_{14} suppress a wide variety of macrophage and neutrophil responses to LPS.[17] Although soluble CD_{14} appears to mediate LPS-induced activation of endothelial cells, excess soluble CD_{14} inhibits the release of cytokines from macrophages. Recombinant human CD_{14} is also being investigated as therapy for sepsis. LBP as a potential target for therapy is also being investigated.

c) LPS neutralising proteins

A number of endogenous neutrophil proteins that can bind and neutralize LPS have been described.

Bactericidal permeability-increasing (BPI) protein, a protein that has significant amino acid sequence homology with LBP, has shown promise in preclinical studies of gram-negative sepsis. BPI neutralizes many biological effects of LPS and, by a separate mechanism, also exerts a cytotoxic effect on some species of gram-negative bacteria. Studies with experimental animals have shown that this protein is protective against endotoxaemia.[18] Other neutrophil-derived LPS-binding proteins include CAP-18 and P-15.[19,20]

Polymyxin B is one of a group of polycationic antibodies with LPS-neutralizing capacity. Polymyxin binds to the lipid A portion of LPS and inhibits LPS responses *in vitro*.[21] Clinical use has been limited by its systemic toxicity.

d) Lipid A analogues

A number of precursors and analogues of lipid A have been investigated as competitive endotoxin antagonists. Monophosphoryl lipid A (MPL), a LPS analogue, displays reduced toxicity while retaining the adjuvant and endotoxin-inducing properties of LPS. In phase 2 clinical studies, MPL was generally well tolerated. Administration of MPL to patients with gram-negative sepsis might be of value.[22]

(2) Strategies Directed Against Host-derived Inflammatory Mediators

Extensive studies in animals and humans indicated that interleukin-1 and TNFα were top contenders as the principal toxic secondary mediators and it seemed logical to develop and evaluate molecules that specifically blocked these two cytokines.

a) Cytokines

TNFα is a potent inflammatory cytokine released by macrophages and neutrophils in response to various stimuli including endotoxin and gram-positive bacteria. TNFα circulates as a molecular trimer and because of this it is able to generate clustering of its receptors on cell

surfaces, leading, after binding, to intracellular signal transduction. Any therapy against TNFα must bind at least 2 of the 3 components of the TNFα trimer to be effective.

Two monoclonal antibodies to human tumour necrosis factor (MAbs to TNFα) have been tested in large-scale clinical trials. In a recent double-blind placebo-controlled trial, murine MAb to human TNFα was reported to have no overall benefit in patients with severe sepsis but there was a significant reduction in mortality 3 days after therapy in septic shock patients.[23] A phase 3 placebo-controlled trial of another murine MAb to TNFα (Bay x 1351) documented no overall difference in mortality in 533 patients with severe sepsis who received MAb (two dosing regime) or placebo.[24] Among a subset of 247 patients who survived 28 days, there was significantly more rapid reversal of shock in both treatment groups compared with placebo.

An alternative approach is the use of soluble TNFα receptors (sTNFRs), which occurs in 75-kD (type 2) and 55-kD (type 1) forms and may modify the response to endogenous TNFα during sepsis. A recent study in patients with septic shock treated with a dimeric form of type 2 TNF receptor linked with Fc portion of IgG1 (TNF:Fc) suggested that mortality was higher among treated patients than among placebo recipients.[25]

The effect of IL-1 can be blocked by a naturally occurring 23-kD protein, IL-1 receptor antagonist (IL-1ra). A recombinant form of IL-1ra was reported to reduce mortality from endotoxic shock in rabbits.[26] Similarly, a prospective open-label placebo-controlled phase II trial in 99 patients using 3 different doses of IL-1ra suggested a dose-dependent survival benefit at 28 days.[27] A multicentre randomised placebo-controlled trial in 893 patients with sepsis syndrome did not demonstrate a survival advantage for patients treated with IL-1ra.[28] A retrospective analysis of the data demonstrated a predicted risk of mortality of 24% or greater and among patients with dysfunction of one or more organs.

b) Corticosteroids

Corticosteroids inhibit a variety of inflammatory responses including macrophage activation by endotoxin. In animal models, pre-treatment with corticosteroids was protective against endotoxaemia. In 1987, 2 large multicentre controlled trials of high-dose glucocorticoid therapy given early in severe sepsis and septic shock failed to demonstrate any benefit in reducing mortality in this patient population.[29,30] Pharmacologic doses of corticosteroids are no longer recommended for the treatment of septic shock.

c) Lipid mediators

The platelet activating factor (PAF) is a potent inflammatory molecule with pleiotropic effects on a variety of cells, including neutrophils, endothelial cells and platelets. In a randomised, placebo-controlled trial of the PAF antagonist BN 502021 in patients with severe sepsis, mortality at 28 days was 51% in the placebo group and 42% in the treated group.[31]

The role of arachidonic acid metabolites in septic shock is still unclear. Overall thromboxanes and leukotrienes have deleterious effects in sepsis, while prostaglandins (especially PGE_1 and PGI_2) may be beneficial by inducing vasodilatation, reducing procoagulant activity and improving tissue oxygenation in critical organs.

In animal models, inhibitors of cyclogenase pathway e.g. ibuprofen moderated the toxicity of endotoxin and TNFα or IL-1. A multicentre randomised placebo-controlled trial of intravenous ibuprofen in 455 patients with sepsis had no effect on survival or the development of shock or adult respiratory distress syndrome (ARDS).[32]

Inhibitors of thromboxane may be preferable to inhibitors of cyclogenase in the treatment of sepsis because prostaglandin synthesis is preserved in the former. In a recent placebo-controlled trial of the antifungal agent, ketoconazole (a thromboxane synthetase inhibitor), there was statistically significant reductions in the rate of development of ARDS and in the 30-day mortality.[33] These promising findings need to be confirmed in larger trials.

d) Nitric oxide

Nitric oxide (NO) is an important endogenous vasodilator that is produced from L-arginine by the enzyme nitric oxide synthase. Methylene blue, an inhibitor of nitric oxide action, has been shown to improve haemodynamics by increasing myocardial function and oxygen delivery.[34]

A calcium-independent form of nitric oxide synthase can be induced by sepsis and some inflammatory mediators (inducible NOs). It has been suggested that inhibition of nitric oxide synthase might be beneficial in the treatment of septic shock.[35] The result of nitric oxide synthase inhibition in animal models is conflicting and there is evidence that this inhibition may be harmful in animal models.[36] In the first reported clinical application of N-monomethyl-L-arginine treatment of 2 patients was accompanied by a rise in blood pressure, 1 patient died and 1 survived.[37] A large multi-centre trial of nitric oxide inhibition in patients with septic shock is currently ongoing.

(3) Strategies Designed to Limit Tissue Damage

Much of the tissue injury that complicates sepsis results from the migration of activated neutrophils into tissues followed by the release of destructive neutrophil

enzymes and reactive molecules. The various targets at which this process could be interrupted include:

- neutrophil chemotaxis e.g. monoclonal antibody to C5a and IL-8,
- neutrophil adherence to endothelium e.g. monoclonal antibody to selectin,[38]
- antioxidants and free radical scavengers e.g. superoxide dismutase, allupurinol, N-acetylcysteine and catalase, and
- protease inhibitors e.g. aprotinin, antithrombin III, hirudin, anti-elastase.

The use of plasma exchange procedures (including high volume haemofiltration) to remove such endotoxins, cell debris as free myoglobin and haemoglobin, and excessive amounts of cytokines, has been successfully tried in smaller studies since 1984. These studies showed a survival rate of 75% by adding such therapeutic interventions to the conventional therapy.[39,40]

Further clinical studies are needed to determine the therapeutic potential of these various agents.

Conclusion

Despite the remarkable number of clinical trials performed in the past decade to evaluate drugs for the treatment of sepsis, emerging adjunctive therapies has not yet altered the course of this catastrophic illness. Sepsis is the clinical manifestation of multiple component processes, each of which may optimally require separate interactions directed at that process. How these processes interact with one another to benefit or injure the host is less well understood.

The logical progression of research would be to combine different therapeutic agents. Further studies are required to define the most effective combination therapy. In addition, the development of diagnostic tests that determine groups of patients likely to respond to specific types of adjunctive therapy would be of great value in the selection of treatment.

Thus, adjunctive therapy if they are to be effective, may have to be given early or perhaps prophylactically administered to patients identified to be at high risk.

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