Adjunctive Therapy for Septic Shock: A Review of Experimental Approaches

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Septic shock remains a major cause of morbidity and mortality, especially in the intensive care setting. A vast array of treatment strategies is under investigation; despite success in animal models, no effective adjunctive therapy has yet been approved for clinical use. This paper reviews the development of experimental therapies for sepsis and discusses those treatments that show promise for application in humans. Approaches to treatment fall into three broad categories: strategies directed against bacterial components, those directed against host-derived inflammatory mediators, and those designed to limit tissue damage. Because septic shock is a dynamic and evolving condition, different strategies may be needed at different stages in the pathogenesis of sepsis. Through carefully performed trials and thoughtful selection of combination therapy aimed at different points in the pathological process, it may be possible in the future to modify the course of this serious condition.

Despite advances in antimicrobial therapy and medical support, septic shock remains a major cause of morbidity and mortality among hospitalized patients. In fact, the prevalence of septic shock has been increasing during the past 30–40 years, with an estimated 400,000 cases and 100,000 deaths per annum in the United States. The most familiar association is that between gram-negative bacterial infections and septic shock, but the sepsis syndrome clearly can involve gram-positive bacteria or indeed almost any class of infecting organism [1, 2]. Mortality attributable to established septic shock has changed little over the past 20 years. As our ability to sustain critically ill patients continues to improve, the development of new therapeutic strategies for septic shock remains a substantial challenge.

The last few years have seen an enormous proliferation of novel agents that have been developed because of their potential value in the treatment of septic shock. Many such agents take advantage of our increased knowledge of the immunology of the host-bacterial interaction in sepsis; these drugs can be regarded as immunotherapies. Other products are more conventional pharmaceuticals but are not antimicrobial agents in the usual sense of the term. To encompass all of these new approaches, we use the term "adjunctive therapies." The extraordinary diversity of these adjunctive therapies and the great interest in this field make it difficult to keep abreast of developments. In this paper we draw together the various strands of information on this subject and provide a framework for future discussion. We focus on treatments that modify the host's response to infection; we do not consider antibiotic therapy, cardiovascular strategies, or approaches aimed at reducing the risk of infection in the intensive care setting.

The pathogenesis of septic shock has been reviewed in detail [1, 2] and herein is discussed only in relation to potential targets of therapy. Because many different strategies have been tried, it is not feasible to discuss them all in detail. Thus we focus our attention on the principles behind intervention and on the specific treatments that have been most thoroughly investigated.

Clearly, as is illustrated in figure 1, septic shock is the end result of the interaction of numerous pathways after the activation of inflammatory cells exposed to bacterial products. Potential targets of treatment for sepsis may be usefully considered in relation to three main mechanisms.

(*1*) *Prevention of host cell activation.* Therapy targeting the initial interaction of bacterial products (notably, gram-negative bacterial endotoxin) with inflammatory cells is likely to be of most benefit when administered either early in the pathogenesis of sepsis—before widespread vascular injury has taken place—or prophylactically to high-risk patients. However, since it is important to prevent further activation of inflammatory cells during the course of bacterial sepsis, there may be a role for such treatment even after shock has become established. One drawback of such approaches is that they are specific to a single class of organisms (e.g., antiendotoxin therapy for gram-negative bacterial infections). It is often impossible to predict the identity of the infecting organism when patients present with sepsis; therefore, a proportion of patients with other infections will receive an inappropriate drug unless techniques for rapid diagnosis improve.

(*2*) *Inhibition of secondary mediators.* Other therapeutic regimens target secondary inflammatory mediators, such as tumor necrosis factor α (TNF-α). These mediators probably

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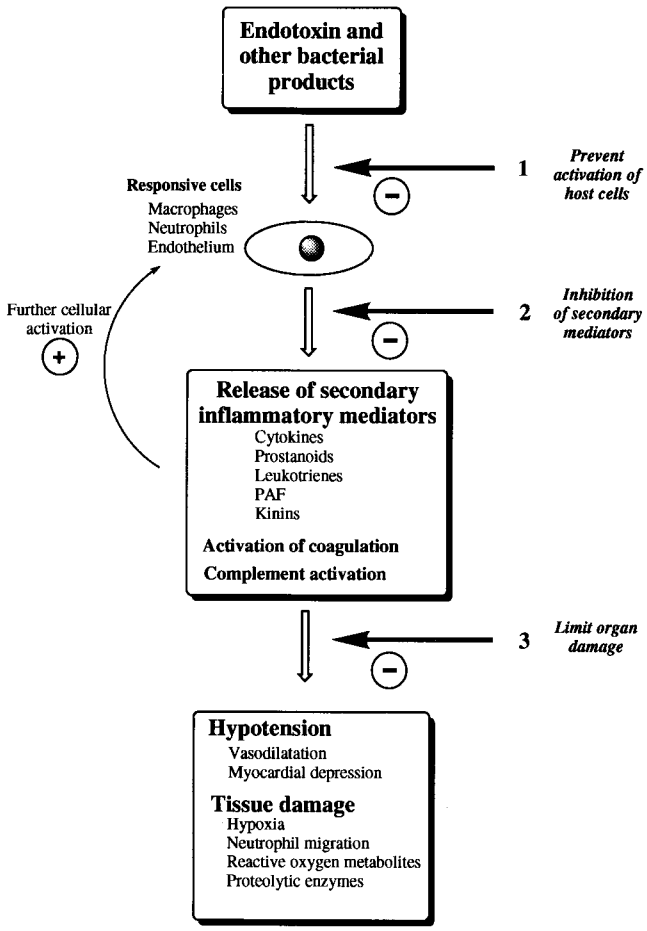

Figure 1. Potential sites of action in adjunctive therapies for septic shock. PAF = platelet-activating factor.

play a role (albeit varying in degree) in all forms of sepsis and other inflammatory conditions; thus their blockade could have a wide application. Much research has focused on the search for a single key mediator of sepsis. It is apparent from figure 1 that this concept is flawed: multiple mechanisms can lead to septic shock, and several pathways may need to be inhibited if a therapeutic response is to be attained. However, certain inflammatory mediators, such as TNF-α and interleukin (IL) 1, are of particular importance and stand out as potential therapeutic targets. One cautionary note applies here: an effective immune response is needed to deal with infection, and a balance must be maintained between the inhibition of excessive host responses and the abolition of essential defenses.

(*3*) *Limitation of organ damage.* Finally, it is possible to inhibit some of the pathological processes leading to organ failure in septic shock. Refractory hypotension and widespread endothelial damage with "capillary leak" are the hallmarks of severe sepsis. The migration of activated, primed inflammatory cells—particularly neutrophils—into healthy tissues distant from the site of infection is involved in the pathogenesis of such tissue damage (as, for example, in the adult respiratory distress syndrome, or ARDS) [1, 3]. With the elucidation of the molecular and cellular bases for these events, therapeutic intervention is now feasible.

Antiendotoxin Therapy

Endotoxin (lipopolysaccharide, LPS) is a potent diglucosamine-based glycolipid toxin that is unique to the outer leaflet of the gram-negative bacterial cell wall [4]. Endotoxin, and in particular the lipid A component, is sufficient to induce the pathophysiological changes leading to the sepsis syndrome. Within the past few years, considerable progress has been made in understanding the basis of the cellular response to LPS, and a variety of therapeutic strategies have been or will soon be evaluated in clinical trials. Potential sites of intervention in LPS-induced cellular activation are depicted in figure 2.

Antibodies to Endotoxin

Antibodies to the outer polysaccharide (O antigen) of LPS are highly protective against infection and shock but are specific to the individual bacterial strain. In the early 1970s,

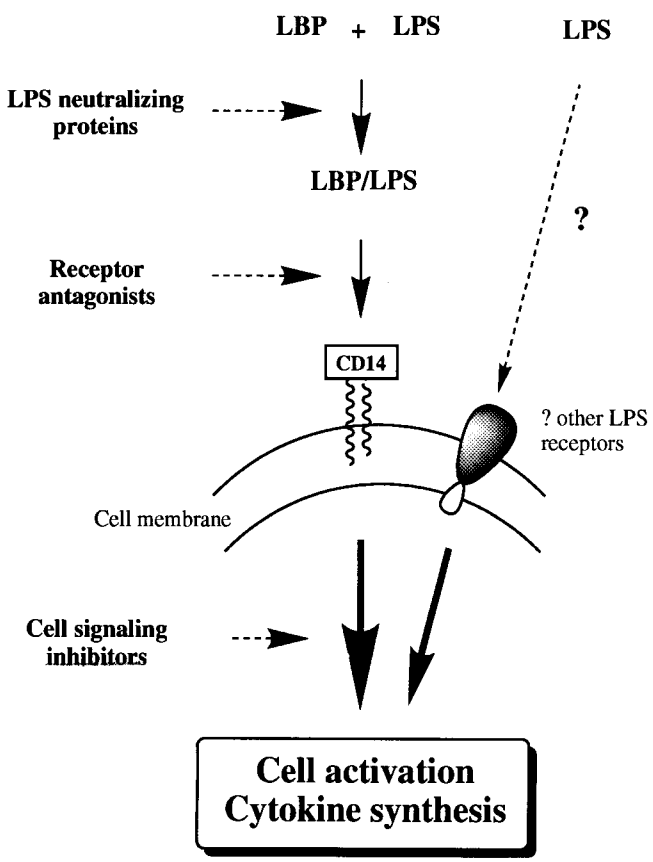

Figure 2. Potential sites for inhibition of cellular responses to endotoxin. LPS = lipopolysaccharide; LBP = LPS-binding protein.

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studies conducted with animals by McCabe and Greely [5] and by Braude and Douglas [6] led to the conclusion that active or passive immunization with antibodies to common components of the core region of LPS was protective against challenge with endotoxin and a variety of heterologous gram-negative bacteria [5, 6]. The protective activity of cross-reactive antibodies was found to reside mainly in the IgM antibody fraction. Subsequently, polyclonal human antisera with high levels of cross-reactive antibodies to LPS were raised either through the vaccination of volunteers with bacteria deficient in the outer-cell-wall polysaccharide or through the selection of blood-donor sera with high concentrations of antibody to LPS [7]. In initial studies involving patients, such antisera appeared to reduce mortality from gram-negative bacteremia [7, 8]. Furthermore, prophylactic administration of an antiserum to LPS reduced mortality from gram-negative sepsis among surgical patients [9]. Studies with immunoglobulin preparations containing IgG rather than IgM activity against LPS failed to demonstrate a consistently protective effect of LPS antisera; under certain circumstances, in fact, standard immunoglobulin appeared to be of equal or greater benefit in reducing infection-related mortality in high-risk groups [10]. As a result of these problems and difficulties in establishing an adequate supply of human antisera, researchers' interest shifted toward monoclonal antibodies (MAbs) to cross-reactive LPS epitopes.

Modern MAb technology has made possible the production of large amounts of pure antibodies to LPS. Experience with these compounds highlights the difficulties inherent in developing and testing treatments for critically ill patients with sepsis. Two MAbs have been the subject of large-scale clinical trials and some controversy [11–13]. HA-1A (Centoxin™, Leiden, the Netherlands) is a human IgM MAb derived from the spleen of a patient vaccinated with the *Escherichia coli* rough mutant J5 [14]. This antibody binds to LPS and lipid A, probably at an epitope different from that to which MAb E5 binds [15]. HA-1A is not an LPS-neutralizing antibody in vitro; its mechanism of action has not been defined but may be related to enhanced clearance of LPS/HA-1A/complement immune complexes via complement receptor (CR) type 1 [16]. The initial phase 3 trial included 543 patients with sepsis and demonstrated no overall benefit of HA-1A, which was compared with human serum albumin placebo; however, HA-1A appeared to afford significant protection to a subgroup of 200 patients with gram-negative bacteremia [11]. The results of a second placebo-controlled study have now been presented. In this trial, 2,199 patients were enrolled, of whom 621 (28%) had gram-negative bacteremia. The overall mortality was 33% among patients with gram-negative bacteremia who received HA-1A and 32% in the placebo group. Among patients without gram-negative bacteremia, the corresponding mortality figures were 41% and 37% ($P = .134$). Thus this study documented a lack of overall clinical benefit of HA-1A and a nonsignificant survival disadvantage among patients without gram-negative bacteremia (C. Sprung, personal communication). As a result, further development of HA-1A as a therapeutic agent for septic shock was halted, apart from a placebo-controlled trial in children with meningococcal infection.

Neither of two trials with murine MAb E5 (Xoma, Berkeley, CA) showed an overall benefit in terms of survival. In the first trial of E5, treatment appeared to enhance the rate of survival among patients with gram-negative sepsis who were not in shock [12]. This finding was not confirmed by the second study, which did demonstrate, however, a trend toward improved survival among treated patients with major organ failure [17]. A third phase 3 trial of E5 has begun and will include only patients with gram-negative bacteremia.

Until further data become available, neither HA-1A nor E5 can be recommended for the treatment of gram-negative sepsis. Although the experience with these MAbs has been disappointing, the results do not preclude the development of an effective LPS-neutralizing antibody of clinical benefit in the future. A number of other cross-reactive antibodies to LPS have been described (including T88, SDZ 219-800, SDJS 1.17.15, 8-2/26-20, D6B3, MLA-1, and GL11) but have not yet been tested in large clinical trials [18, 19]. Cross-reactive antibodies to LPS, as described above, would be most widely applicable to patients with gram-negative sepsis, but antisera or MAbs have also been raised to specific pathogens such as *Klebsiella* and *Pseudomonas* species, and these preparations may prove to have a therapeutic role under certain circumstances [20–22].

Inhibition of Endotoxin-Induced Cellular Activation

Elucidation of the elements involved in cellular signaling and activation by endotoxin has been a major goal of LPS research and is a necessary first step in the design of rational antiendotoxin therapy. Figure 2 illustrates the points at which the activation of cells by LPS could be inhibited. A number of specific LPS receptors on the surface of LPS-responsive cells mediate cellular activation, opsonophagocytosis of gram-negative bacilli, and uptake and detoxification of LPS. The best-characterized and probably most physiologically relevant of these receptors is the glycosylphosphoinositol-linked protein CD14 found on macrophages and neutrophils. LPS binds to a serum protein, LPS-binding protein (LBP), and the LPS/LBP complex binds to CD14 [23, 24]. In this context LBP appears to act as a carrier protein, increasing by 100- to 1,000-fold the sensitivity of CD14-bearing cells to LPS. The mechanism by which cellular signaling occurs after CD14 binding is unclear but may be a target for therapy. In addition, some CD14-negative cells (e.g., endothelial cells) can respond to LPS in a serum-dependent manner because circulating CD14 (soluble CD14) is involved in signaling to these cells [25].

Inhibition of LPS/LBP binding to cells by MAbs to CD14

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suppresses a wide variety of macrophage and neutrophil responses to LPS [24, 26, 27]. These MAbs also inhibit LPS-induced activation of endothelium, a process that is thought to be mediated by soluble CD14 [25]. As yet, few data are available regarding CD14 blockade in models of sepsis, but CD14 is obviously an important potential target of therapy, and relevant studies are in progress. Although soluble CD14 appears to mediate LPS-induced activation of endothelial cells, excess soluble CD14 inhibits the release of cytokines from macrophages, and recombinant human CD14 is also being investigated as therapy for sepsis. Antibodies to LBP inhibit cellular responses to low concentrations of LPS in vitro and protect mice against lethal challenge with LPS or lipid A [28]; more extensive studies of LBP as a potential target for treatment are awaited.

LPS-Neutralizing Proteins

A number of endogenous neutrophil proteins that can bind and neutralize LPS have been described. One of these, bactericidal/permeability-increasing protein (BPI), has shown promise in preclinical studies of gram-negative sepsis. BPI is a 55- to 60-kD neutrophil primary granular protein exhibiting 45% sequence identity with LBP [29, 30]. BPI has a higher affinity for LPS than does LBP and thus can compete with LBP for binding to LPS [30, 31]. BPI neutralizes many biological effects of LPS and, by a separate mechanism, also exerts a cytotoxic effect on some species of gram-negative bacteria [32]. These characteristics make BPI an attractive agent for the treatment of gram-negative sepsis. The LPS-neutralizing activity of BPI resides in the 25-kD N-terminal portion of the molecule, whose expression as a recombinant 23-kD N-terminal fragment has allowed large-scale production of pure protein [33]. Studies with animals have shown that this protein ($rBPI_{23}$) is protective against endotoxemia in mice and rats [34] and in murine models of *E. coli* sepsis and pneumonia [35, 36]. The relative contributions of the antibiotic and endotoxin-neutralizing properties of $rBPI_{23}$ to its protective effect in these models are uncertain, and the range of gram-negative pathogens against which $rBPI_{23}$ is active in vivo has not been defined. $rBPI_{23}$ has a short half-life and therefore will need to be administered by continuous infusion. Clinical studies have not yet been conducted, but a large-scale multicenter trial is being planned. Fusion chimeras of LBP and BPI have also been constructed; these proteins are able to neutralize endotoxin but have a longer circulating half-life than BPI. One of these preparations—a fusion protein including residues 1–199 of LBP and amino acids 201–245 of the C terminal of BPI—has shown protective activity against LPS challenge of animals [37]. Whether these chimeric proteins will be better LPS antagonists than BPI remains to be proven.

A number of other neutrophil-derived LPS-binding proteins have been described, such as CAP-18 [38] and P-15 [39]. Naturally occurring LPS-neutralizing proteins include those derived from horseshoe crabs (*Limulus polyphemus* and *Tachypleus tridentatus*) [40, 41]. These proteins inhibit LPS responses in vitro. The recombinant 11.8-kD protein from *L. polyphemus* (termed endotoxin-neutralizing protein) has also been shown to protect rabbits from *E. coli* sepsis and meningococcal shock and will be studied in humans [40]. The currently available data are insufficient for an assessment of the potential role of these agents in the treatment of sepsis.

After incubation in plasma or serum, endotoxin becomes less toxic because of its adsorption into complexes with serum proteins or lipoproteins [42]. These proteins may provide a degree of natural protection from endotoxemia by sequestering LPS away from LBP and CD14. Low- and high-density lipoproteins and apolipoprotein A-1 inhibit LPS-induced release of cytokines from macrophages [43, 44]. Very-low-density lipoproteins and chylomicrons have also been shown to protect mice from a lethal endotoxin challenge [45]. The iron-binding protein lactoferrin binds LPS and inhibits LPS-induced priming of neutrophils [46]. Thus far, none of these agents has been evaluated in large-scale clinical trials.

Polymyxin B is one of a group of polycationic antibiotics with LPS-neutralizing capacity. This compound binds to the lipid A portion of LPS, inhibits LPS responses in vitro, and protects animals from endotoxemia [47, 48]. The clinical use of polymyxins has been limited by their systemic toxicity. A derivative of polymyxin B, polymyxin B nonapeptide, is less toxic but is also a weaker LPS inhibitor than the parent compound [49]. Extracorporeal removal of endotoxin from plasma by absorption to polymyxin B has been tried with some success in animal models [50].

Lipid A Analogues

Lipid A is a unique acylated diglucosamine bisphosphate that forms the backbone of almost all bacterial LPS. Changes in the structure of lipid A can markedly alter toxicity, and a number of precursors and analogues of lipid A have been investigated as competitive endotoxin antagonists (reviewed in [51]).

The first lipid A precursor identified was the monosaccharide lipid X. Lipid X was protective against endotoxemia in some animal models and inhibited LPS-induced priming of neutrophils [51]. However, lipid X appears to be a relatively weak LPS antagonist in vitro. Moreover, in a recent study, highly purified lipid X afforded no protection in a canine model of sepsis [52].

Diglucosamine-based lipid A analogues are more potent than lipid X as LPS antagonists. These analogues will be considered together herein because their biological effects on human cells are similar. The best-characterized disaccharide LPS inhibitors are lipid 406 (lipid IVa, lipid Ia, or LA-14-

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PP), LPS deacylated by the neutrophil granular protein acyloxyacylhydrolase (dLPS), lipid A from *Rhodopseudomonas sphaeroides*, and LPS from *Rhodobacter capsulatus*. These molecules have the same diglucosamine backbone as lipid A but differ from it in the number and/or length of the acyl side chains. Their inhibitory effects are specific to lipid A, LPS, or gram-negative bacteria, with a 5- to 10-fold excess (wt/wt) of the antagonists required to completely block the effects of LPS on macrophages, neutrophils, and endothelial cells [51]. Studies with animals have been limited by the lack of sufficient quantities of pure lipid. However, it has been shown that pretreatment with *R. sphaeroides* lipid A protects mice from lethal endotoxemia [53]. In addition, this antagonist appears to retain the ability to induce tolerance to subsequent (48-hour) challenge of rats with LPS [54]. Administration of these LPS antagonists to humans would be difficult because of the large infusions of lipid that would be required. However, a recently described synthetic lipid A analogue, E5331 (structure not available), is a much more potent LPS antagonist and will soon be studied in humans [55].

Monophosphoryl lipid A (MPL) is another LPS analogue that has been investigated as a therapeutic agent in sepsis. Unlike the compounds described above, MPL is not an LPS antagonist. Rather, it displays reduced toxicity while retaining the adjuvant and endotoxin tolerance–inducing properties of LPS. Pretreatment of mice and pigs with MPL does reduce the hemodynamic effects, cytokine levels, and mortality associated with subsequent endotoxin or bacterial challenge [56]. The effects of this early endotoxin tolerance are manifest within 24–48 hours; thus the administration of MPL to patients at high risk of gram-negative sepsis might be of value. In phase 1 clinical studies, MPL was generally well tolerated [57].

Inflammatory Mediators as Targets in Therapy for Septic Shock

To date, no inhibitor of any secondary mediator in septic shock has conferred an unequivocal benefit to patients in controlled trials. However, a number of possible targets for therapy with such agents exist, and many inhibitory compounds have been investigated in vitro or in animal models. As it is not possible to review the results obtained with all of these drugs, only the areas that have been investigated in most detail are discussed herein. Table 1 lists potential therapeutic agents and their targets.

Cytokines

Many different cytokine responses are generated during bacterial sepsis, either as the direct result of activation of macrophages, neutrophils, and endothelial cells or by other inflammatory mediators. Some cytokines, such as TNF-α, IL-1, IL-6, IL-8, and interferon (IFN) γ, are proinflammatory; others, such as IL-4, IL-10, IL-13, and transforming growth factor (TGF) β, have counterregulatory activities.

TNF-α. TNF-α is a potent inflammatory cytokine released by macrophages and neutrophils in response to a variety of stimuli, including endotoxin, gram-positive bacteria, and some bacterial exotoxins [58]. TNF-α is released in humans after endotoxin injection, and high initial levels of TNF-α have correlated with a poor clinical outcome in some but not all studies of patients with septic shock. Antibodies to TNF-α are protective in some animal models of endotoxemia and gram-negative bacteremia [59–61]. MAbs to TNF-α have also been studied in animal models of gram-positive infection. An MAb to TNF-α protected against *Staphylococcus aureus*–induced shock in primates [62], but a different MAb to TNF-α was ineffective in a murine model of *Streptococcus pyogenes*–induced shock [63]. MAbs to TNF-α exhibit some efficacy when administered after bacterial challenge, although protection is maximal only when the antibodies are administered at the same time as the bacteria. The inhibition of TNF-α is not always beneficial: treatment with MAbs to TNF-α has resulted in increased mortality in animal models of localized peritoneal infection with *E. coli* [64].

A number of MAbs to TNF-α have been investigated; two such antibodies have been tested in large-scale clinical trials. In phase 1/2 trials involving patients with severe sepsis, a murine MAb to human TNF-α (CB0006) was well tolerated despite the development of antibodies to murine proteins [65, 66]. No overall benefit of treatment was evident, but a trend toward benefit (in comparison with historical controls) was noted among patients with high levels of TNF-α at enrollment. Preliminary data from a phase 3 trial of another murine MAb to TNF-α (Bay $\times$ 1351) have been published [67]. In this trial 971 patients who developed sepsis—with or without shock—received this MAb (7.5 or 15 mg/kg) or placebo. No overall difference in mortality was apparent at 28 days; mortality was 33.1% in the placebo group. Although this result was disappointing, an early difference in the rate of survival was detected on day 3: mortality was 33% lower among patients receiving 7.5 mg of MAb/kg than among controls. The protective effect on day 3 was most prominent among patients with shock at presentation, among whom mortality was reduced by 48.7%. The several other MAbs to TNF-α described as potential therapeutic agents in sepsis include MAK 195F [68] and two "humanized" antibodies, cA2 [69] and CDP571 [70]. These results are encouraging, but further clinical studies are needed to define the role of MAbs to TNF-α in the treatment of sepsis.

An alternative approach to the inhibition of TNF-α is the use of soluble TNF-α receptors (sTNFRs). The TNF-α receptor occurs in 75- and 55-kD forms; circulating forms of both receptors are found in the serum of septic patients and may modify the response to endogenous TNF-α during sepsis. These molecules appear to act as both TNF-α carriers

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Table 1. Major secondary targets in the treatment of sepsis.

Potential target or approach	Experimental therapeutic agent(s)
Inhibition of cytokine synthesis/release	Phosphodiesterase inhibitors
	IL-4, IL-10, IL-13, and TGF-β
	Corticosteroids
Specific anticytokine therapy	
TNF	MAbs to TNF, soluble TNF receptors
IL-1	IL-1 receptor antagonist
	MAb to IL-1 receptors
IL-6	MAb to IL-6
IFN-γ	MAb to IFN-γ, soluble IFN-γ receptors
Coagulation cascade	Various agents*
Complement activation	MAb to C5a
	C1-Esterase inhibitors
	Soluble CR1
PAF	PAF receptor antagonists
Arachidonate metabolism	Cyclooxygenase inhibitors
	Lipoxygenase inhibitors
	Leukotriene antagonists
	Thromboxane A_2 inhibitors
	Prostaglandin infusion
Nitric oxide	Nitric oxide synthase inhibitors
Physical removal of mediators	Plasmapheresis and hemofiltration and adsorption of LPS

NOTE. Abbreviations: IL = interleukin; TGF = transforming growth factor; TNF = tumor necrosis factor; MAb = monoclonal antibody; IFN = interferon; CR = complement receptor; PAF = platelet-activating factor; and LPS = lipopolysaccharide.

* See table 2.

and TNF-α antagonists and offer protection against endotoxemia and *E. coli* sepsis [71]. To prolong the circulating half-life of sTNFRs, chimeric molecules have been engineered with dimers of the receptors attached to the Fc portion of human IgG (sTNFR:Fc); these constructs are also effective TNF-α antagonists in certain models of sepsis [71, 72]. However, in a murine model of *E. coli* sepsis, p75 sTNFR:Fc failed to prevent TNF-mediated deaths despite a reduction in peak TNF-α levels and in early mortality [73]. Disappointingly, according to a 1993 press release from Immunex Corporation in Seattle, a phase 2/3 clinical trial with p75 sTNFR:Fc suggested that mortality was higher among treated patients than among placebo recipients. Clinical studies with the p55 sTNFR:Fc construct are due to start in the near future.

IL-1. IL-1 is an important secondary mediator in sepsis and could be inhibited by a number of approaches [74]. For example, an inhibitory MAb to the IL-1 receptor afforded some protection from endotoxemia in mice [75]. Alternatively, IL-1 release may be prevented through inhibition of its activation by IL-1β-converting enzyme [74]. Most studies of the inhibition of IL-1 in sepsis have focused on the IL-1 receptor antagonist (IL-1ra), a 23-kD protein initially isolated from the urine of febrile patients and the culture supernatant of activated macrophages. A substantial (~1,000-fold) excess of IL-1ra is needed to abolish the response to IL-1. In studies with animals, native and recombinant IL-1ra reduced mortality from endotoxemia and appeared to be active even when given after endotoxin challenge [76, 77]. Furthermore, in a rabbit model of gram-positive infection, IL-1ra reduced rates of *Staphylococcus epidermidis*–induced hypotension and death [78].

Initial results with recombinant IL-1ra in humans were promising. A phase 2 study of 99 patients with sepsis syndrome demonstrated that the agent was well tolerated and conferred a dose-dependent survival benefit at 28 days [79]. In contrast, a multicenter placebo-controlled trial involving 893 patients showed no overall beneficial impact of recombinant IL-1ra on 28-day mortality, with figures of 34% and 29% in the placebo and treated groups, respectively [80]. A retrospective analysis of subgroups of patients suggested that, as the severity of disease increased, a significant protective effect of IL-1ra emerged; for example, mortality was decreased by 22% among patients with a predicted mortality of >24%. This observation requires confirmation in further studies.

Other cytokines. IFN-γ enhances macrophage responses to endotoxin and is also synergistic with many other cytokines in inflammatory responses. MAbs to IFN-γ protect mice from endotoxemia and *E. coli* sepsis [81], and this effect is potentiated by the simultaneous administration of MAbs to TNF-α [82]. Unlike the latter antibodies, MAbs to IFN-γ were protective in a murine model of *E. coli* peritonitis [83]. Soluble IFN-γ receptors also inhibit IFN-γ in mice but have not been investigated in models of sepsis [84, 84a].

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High levels of IL-6 are predictive of death in sepsis, and MAbs to IL-6 modify the inflammatory response to endotoxin in chimpanzees [85]. IL-8 is also a potential target of therapy for sepsis; an MAb to IL-8 inhibited neutrophil-mediated lung injury following pulmonary ischemia [86]. No data are available on antibodies to IL-6, IL-8, and IFN-γ in humans. A recently described pituitary-derived cytokine, macrophage migration inhibitory factor, appears to contribute to the lethality of endotoxemia in mice [87]. Clearly, this and other novel cytokines are potential targets of treatment for sepsis.

Protective effect of cytokines. Although TNF-α and IL-1 are implicated in the pathogenesis of sepsis, sublethal injections of these cytokines protect animals against subsequent bacterial challenge in a manner analogous to the induction of tolerance by endotoxin [88, 89]. Differentiation factor/leukemia inhibitory factor, a glycoprotein mediator of leukocyte maturation, is also protective against LPS challenge when administered 2–24 hours beforehand and is synergistic with TNF-α and IL-1β [90]. The protective effects of low-dose cytokines have not yet been explored in humans.

IL-4, IL-10, and IL-13 are potent anti-inflammatory agents and inhibit macrophage activation by LPS [91–93]. The administration of IL-10 protects experimental animals against sepsis and is being evaluated in phase 2 clinical trials [92]. TGF-β inhibits the adherence of neutrophils to endothelium and may also confer a benefit in sepsis by limiting neutrophil-mediated damage to organs [94].

Corticosteroids and Opiate Antagonists

Corticosteroids inhibit a variety of inflammatory responses, including macrophage activation by endotoxin. In animal models, pretreatment with corticosteroids was protective against endotoxemia; in humans, initial studies yielded promising results [95]. Large-scale multicenter trials, however, demonstrated an increase in morbidity and mortality among septic patients given large doses of corticosteroids [96, 97]. Similarly, early encouraging reports of the effectiveness of opiate antagonists in the treatment of septic shock were not confirmed by carefully controlled studies [98].

Phosphodiesterase Inhibitors

Compounds that increase intracellular levels of cyclic AMP decrease the expression of TNF-α mRNA; the underlying mechanism has not been fully elucidated. A variety of compounds, including pentoxifylline, aminophylline, amrinone, rolipram, and the xanthine derivative HWA 138, inhibit phosphodiesterase as well as TNF-α responses to endotoxin and other cellular stimuli [99–101]. Of these compounds, pentoxifylline (oxpentifylline) is the best studied. In addition to inhibiting the release of TNF-α, pentoxifylline reduces neutrophil activation, adhesiveness, and degranulation—an effect that may help to limit tissue damage in sepsis [102]. Pentoxifylline increases survival rates in murine endotoxemia and also seems to protect against lung injury in animal models of sepsis [100, 103]. Although no clinical trials including patients with sepsis have been reported, pentoxifylline prevented fever and inhibited the elevation of serum concentrations of TNF-α after endotoxin infusion into volunteers [104].

Table 2. Experimental approaches to the treatment of coagulopathy in sepsis.

Agent	Mechanism of action
α1-Antitrypsin Pittsburgh	Inactivation of thrombin
Antithrombin III	Inactivation of thrombin Inhibition of Factors IXa, Xa, XIa, and XIIa
Bradykinin antagonists	Inhibition of bradykinin
C1-Esterase inhibitor	Inhibition of Factors XI and XII and kallikrein
Monoclonal antibody to Factor XII	Blockade of activation of Factor XII
Fresh-frozen plasma	Replenishment of coagulation factors
Heparin	Binding to and activation of antithrombin III
Hirudin	Inhibition of thrombin
Platelet-activating factor antagonists	Reduction of platelet aggregation
Protease inhibitors	Reduction of contact activation of coagulation
Protein C	Inactivation of Factors Va and VIIa
Streptokinase	Activation of plasminogen
Thrombomodulin	Activation of protein C
Thromboxane inhibitors	Reduction of platelet aggregation
Monoclonal antibody to tissue factor	Blockade of activation of tissue factor
Tissue-factor pathway inhibitor	Inhibition of tissue-factor activation Inactivation of Factor Xa

Coagulopathy and Sepsis

Widespread activation of the coagulation pathways leading to disseminated intravascular coagulation (DIC) is a serious complication of bacterial sepsis for which effective therapy is badly needed. A number of treatment strategies directed at different components of the coagulation/fibrinolytic pathways have been proposed (table 2), but few agents have been examined in clinical trials [105].

Endogenous inhibitors of coagulation. Antithrombin III is a natural inhibitor of both the direct and the contact pathway of coagulation. Levels of antithrombin III fall in sepsis. In animal models of endotoxemia and sepsis, the administration of antithrombin III reduces tissue damage and mortality [106]. Antithrombin III may be beneficial to patients with septic shock complicated by DIC. One small clinical trial documented an apparent benefit of antithrombin III, in con-

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junction with fresh-frozen plasma, in septic patients [107]. Moreover, a placebo-controlled trial of antithrombin III in 35 patients with septic shock and DIC showed a significant improvement in markers of DIC and a nonsignificant reduction in mortality in the treated group [108]. Larger-scale studies are awaited. Levels of protein S and protein C are also reduced in sepsis, and replenishment of protein C modifies the activation of coagulation after challenge of baboons with *E. coli* [109].

Contact system inhibitors. Complement activation and the contact system of coagulation can be inhibited by C1-esterase inhibitor. A small clinical study demonstrated improvement in the condition of four of five septic patients after the intravenous administration of C1-esterase inhibitor, with increased levels of Factor XII, decreased complement activation, and improved hemodynamic function [110]. Specific inhibitors of the contact pathway have been developed, including an MAb to Factor XII, an MAb to tissue factor, and a tissue-factor pathway inhibitor. No data from studies of patients are available, but these agents have been shown to be effective inhibitors of coagulopathy in animal models of sepsis [111–113]. Protease inhibitors, such as aprotinin, also limit contact activation of coagulation and are discussed later.

Heparin and fresh-frozen plasma. In some animal models of sepsis, heparin has improved hemodynamic function and reduced mortality; however, no large-scale clinical trials have been performed [114]. Fresh-frozen plasma contains many of the coagulation factors that are consumed in sepsis, but its use in the treatment of sepsis-associated DIC is controversial. A number of small studies have demonstrated some benefit [107, 115], but these results require confirmation. Furthermore, a recent case-control study of 336 patients with meningococcal septicemia in Norway suggested that the administration of fresh-frozen plasma may actually increase mortality [116]. Thus, in the absence of controlled trials, it is not possible to recommend the use of heparin or fresh-frozen plasma in sepsis-associated coagulopathy.

Bradykinin

Bradykinin is a peptide proinflammatory mediator that may be released during tissue damage and may induce endothelial injury, activation of coagulation, hypotension, and cytokine release. Bradykinin antagonists have shown protective activity in animal models of sepsis [117, 118]. A large multicenter clinical study of one of these antagonists, CP-0127, in the treatment of the systemic inflammatory response syndrome has been completed, but the results are not yet available.

Lipid Mediators

The lipid mediators of interest in sepsis are platelet-activating factor (PAF) and arachidonic acid metabolites.

Platelet-activating factor. PAF is a potent inflammatory molecule with pleiotropic effects on a variety of cells, including neutrophils, endothelial cells, and platelets. A number of natural PAF antagonists exist (e.g., terpenes), and a variety of synthetic PAF receptor blockers have been developed relatively recently [119]. In animal models of both gram-negative and gram-positive bacterial sepsis, PAF antagonists have been shown to alleviate hypotension and decrease mortality [120–122]. A randomized, placebo-controlled trial of the PAF antagonist BN 502021 in 262 patients with severe sepsis has been reported [123]. Among patients with documented gram-negative infections, mortality at 28 days was 57% in the placebo group and 33% in the treated group ($P = .011$). No such differences were detected among patients with gram-positive infections or among those without documented infections. A study aimed at confirming this result is in progress.

Thromboxane, prostaglandins, and leukotrienes. The role of this group of arachidonate metabolites in septic shock is still unclear; current information has recently been reviewed fully by Bone [119]. Thromboxane A_2 and prostaglandins are released via the cyclooxygenase pathway, while leukotrienes are the products of lipoxygenases. Overall, thromboxane 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 [119].

In animal models inhibitors of the cyclooxygenase pathway (e.g., ibuprofen) moderated the toxicity of endotoxin and TNF-α or IL-1 [124, 125]. In volunteers ibuprofen prevented endotoxin-induced fever but not TNF-α release [126]. In a small study of septic patients, hemodynamic variables and temperature were closer to normal after the administration of ibuprofen [127].

Inhibitors of thromboxane may be preferable to inhibitors of cyclooxygenase in the treatment of sepsis because prostaglandin synthesis is preserved in the presence of the former. In a recent placebo-controlled trial of the antifungal agent ketoconazole—a thromboxane synthetase inhibitor—54 patients with sepsis received 400 mg daily by mouth [128]. Statistically significant reductions were noted in the rate of development of ARDS and in subsequent (30-day) mortality. These changes were accompanied by a significant reduction in the level of thromboxane B_2 after the first dose of ketoconazole. However, the inhibition of thromboxane may not be sufficient to account for the observed reduction in rates of ARDS: other specific thromboxane synthetase inhibitors have not been shown to be beneficial in sepsis or ARDS [129]. The promising findings with ketoconazole need to be confirmed in larger trials. Preliminary data from studies with animals suggest that leukotriene inhibitors may afford some protection in sepsis [119]. Further studies are needed to assess the role of the different arachidonate metabolites in sepsis and how these compounds can be manipulated to best advantage.

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Complement Activation

Endotoxin and other bacterial products activate complement by both the direct and the indirect pathways. The activation of complement has been associated with the development of shock in patients with gram-negative bacteremia [130]. In addition, complement components such as C5a are involved in the recruitment of neutrophils to the lung in ARDS. C1-Esterase inhibitor has shown promise in a small clinical trial, as has already been discussed [110]. Recombinant human CR1 inhibits complement activation by both pathways and has recently been shown to be protective against neutrophil-mediated lung injury in animal models [131]. A rabbit MAb to human C5a protected primates from lung injury induced by challenge with *E. coli* [132], but no clinical data are available on therapy with antibody to C5a or soluble CR1.

Physical Removal of Endotoxin and Inflammatory Mediators

One direct approach to the treatment of sepsis is the removal of circulating endotoxin and inflammatory mediators. A full discussion of the various techniques employed in this approach is beyond the scope of this paper. Plasma or whole-blood exchange, hemofiltration, plasmapheresis, leukapheresis, and plasma perfusion through adsorptive columns have all been used experimentally [50, 133–136]. In animal models, hemofiltration or plasma exchange removes cytokines and is associated with an improved outcome. Fewer data (none from large trials) are available on the clinical use of this strategy in sepsis. Small studies of plasma or whole-blood exchange have suggested a benefit to patients with meningococcal septicemia [136], and a recent study has demonstrated that both TNF-α and IL-1 are cleared from the circulation of septic patients by hemofiltration [135]. Further investigation is clearly warranted. Although it will be difficult to perform large-scale multicenter trials, such studies have been undertaken and completed in relation to other diseases—for example, plasmapheresis in the Guillain-Barré syndrome.

Nitric Oxide

Nitric oxide (NO) is responsible for endogenous vasodilator tone and was previously referred to as endothelium-derived relaxant factor. There is considerable evidence that the profound vasodilatation seen in refractory septic shock is mediated, at least in part, by NO. NO synthase exists in constitutive and inducible isoforms. LPS and several of the major inflammatory cytokines seen in sepsis are potent activators of the inducible form [137]. A number of NO synthase inhibitors have been developed and have conferred protection in some animal models of sepsis. However, the inhibition of NO synthase has not proven beneficial in all animal models. In a canine model of sepsis, the inhibition of NO resulted in hemodynamic improvement but also in higher mortality [138]. In rabbits pretreatment with the NO synthase inhibitor *N*-monomethyl-L-arginine (L-NMMA) exacerbated LPS-induced hypotension [139]. The widespread distribution of NO, together with its multiple roles in different tissues, probably explains these variable effects of NO inhibition.

In the first reported clinical application of L-NMMA, treatment of two patients was accompanied by a rise in arterial pressure [140]. In a placebo-controlled, randomized study of 12 patients with sepsis, the same investigators found significant increases in mean arterial pressure and in systemic and pulmonary vascular resistance with the intravenous infusion of 1 mg of L-NMMA/(kg · h) [141]. However, these changes were accompanied by a fall in cardiac output and a reduction in oxygen delivery to tissues. In a separate study of 15 patients with the sepsis syndrome, mean arterial pressure increased from 89 mm Hg to 140 mm Hg after the administration of an NO synthase inhibitor, but this change was accompanied by a fall in cardiac index and a rise in right atrial pressure [142]. In the same study the infusion of L-arginine (the substrate for NO synthase) induced transient hypotension but also a rise in cardiac index and tissue oxygenation.

Thus, the role of NO synthase inhibitors in the treatment of sepsis remains to be defined. The availability of specific inhibitors of inducible NO synthase would help to answer the relevant questions. Such agents are in the early stages of development but have not yet been studied in humans.

Inhibition of Tissue Injury

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 points at which this process could be interrupted are listed in table 3.

Antiadhesion Molecule Therapy

Several groups of specific receptors on the neutrophil and endothelial-cell surface mediate neutrophil margination and endothelial rolling followed by adherence and transmigration at inflammatory sites. These receptors include integrins, lectins, intercellular adhesion molecules, and endothelial leukocyte adhesion molecules. The blockade of neutrophil adherence/migration abrogates tissue damage in a variety of inflammatory situations. MAb 1B4, directed against CD18 (the β subunit of the neutrophil β_2 integrins), prevented tissue damage in animal models of tissue ischemia and meningitis [143, 144]. An MAb to lymphocyte function–associated molecule 1 protected mice from liver damage due to *Propionibacterium acnes* and shock due to *E. coli* [145]. Inhibition of P-selectin-mediated leukocyte adherence to endothelium by oligosaccharides protected rats from cobra venom factor–

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Table 3. Inhibition of tissue damage in sepsis.

Potential target	Experimental therapy
Neutrophil chemotaxis	MAb to C5a
	MAb to IL-8
Neutrophil activation	Phosphodiesterase inhibitors
	Adenosine
Neutrophil adherence to endothelium	MAb (1B4) to CD18
	MAb to ELAM
	MAb to selectin or oligosaccharides
	IL-4 and TGF-β
Antioxidants and free-radical scavengers	Superoxide dismutase
	Catalase
	Allopurinol
	Nitrones
	NADPH oxidase inhibitors
	N-Acetylcysteine
	Desferrioxamine
	Lactoferrin
	Dimethylsulfoxide
	Vitamins C and E
Protease inhibitors	Aprotonin
	Hirudin
	Antithrombin III
	α-1 Antiprotease
	Ulnistatin

NOTE. Abbreviations: MAb = monoclonal antibody; IL = interleukin; ELAM = endothelial leukocyte adhesion molecule; TGF = transforming growth factor; and NADPH = reduced nicotinamide adenine dinucleotide phosphate.

induced lung injury [146]. Peptides derived from pertussis toxin are structurally similar to eukaryotic selectins and may inhibit selectin-mediated cell adhesion to endothelium. These peptides have displayed anti-inflammatory activity in an animal model of meningitis by blocking leukocyte migration into the CSF [147]. Phase 1 clinical trials of an antibody to E-selectin have been completed, and further studies are awaited. Clearly, in the setting of active infection, such therapy would need to be used with caution. However, if organ damage—in particular ARDS—can be prevented, then this form of treatment would be a major advance in the management of sepsis.

Antioxidants and Free-Radical Scavengers

Superoxide derivatives are important mediators of neutrophil-induced tissue injury; antioxidant therapy has recently been reviewed [148]. The wide range of natural and synthetic antioxidants under evaluation includes xanthine oxidase inhibitors (e.g., allopurinol), superoxide dismutase, catalase, NADPH oxidase inhibitors (e.g., adenosine); desferrioxamine, and *N*-acetylcysteine. After endotoxin infusion into mice, treatment with superoxide dismutase and catalase reduced mortality; corresponding clinical studies in sepsis have not been performed [149]. The site of action of antioxidants is intracellular. Both polyethylene glycol polymer and liposomal forms of catalase and superoxide dismutase have been developed to prolong the half-life and enhance the delivery of these agents, but no data are available regarding their use in sepsis. *N*-Acetylcysteine appeared to improve hemodynamic function and to moderate lung injury in endotoxemic sheep but was ineffective in a mouse model of endotoxemia and has not been used in this context in humans [149, 150]. A synthetic group of compounds known as nitrones covalently bind to free radicals to form stable, nontoxic molecules and appear to limit both microcirculatory damage and mortality after endotoxin infusion into rats [151].

Protease Inhibitors

Antiproteases may have a variety of beneficial effects in septic shock, including the reduction of procoagulant activity (e.g., by antithrombin III) and the neutralization of neutrophil proteolytic enzymes. Potential therapeutic agents in this group include hirudin, antithrombin III, eglin C, antielastase, aprotinin, and [Arg15]-aprotinin [152]. Studies with animals have yielded some evidence that protease inhibitors reduce lung injury in endotoxemia and protect dogs from septic shock, but no controlled clinical studies have been conducted [152, 153].

Miscellaneous Therapies

Numerous compounds have been described that modify responses to endotoxin in vitro or in vivo and therefore could be considered as therapeutic agents. Some of these compounds are listed in table 4. For most, the details of the mechanism of action and therapeutic potential are not known.

Summary

Despite improvements in our understanding of the pathological events leading to the sepsis syndrome, adjunctive therapy has not yet altered the course of this catastrophic illness. Multiple therapeutic strategies currently being developed show promise in vitro and in experimental animals. A number of agents are being evaluated in phase 2 or phase 3 trials, but the few regimens assessed so far in large-scale, controlled clinical trials have not conferred an unequivocal clinical benefit. Models of sepsis that accurately predict human responses to therapy must be developed and used in the evaluation of new agents.

Because of the complex nature of the inflammatory pathways involved in sepsis, it may be difficult to establish that the inhibition of any one pathway alone is protective. The logical progression of research would be to combine different

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Table 4. Miscellaneous agents investigated for the treatment of sepsis.

Agent	Proposed mechanism of action	Reference
Adenosine	Inhibition of TNF-α release Inhibition of neutrophil activation	[154]
Chloroquine	Inhibition of arachidonate metabolism?	[155]
Chlorpromazine	Inhibition of TNF-α release	[156]
Cloricromene†	Inhibition of TNF-α	[157]
Dehydroepiandrosterone	Inhibition of TNF-α	[158]
Genistein	Inhibition of tyrosine kinase	[159]
H-7	Inhibition of protein kinase C	[160]
Hydrazine	Unknown (cortisol release?)	[161]
Liposome-encapsulated hemoglobin	Posttranscriptional inhibition of TNF-α	[162]
Magainins	Neutralization of LPS	[163]
Magnesium–adenosine triphosphate	Unclear (restoration of intracellular ATP?)	[164]
Estrogen	Inhibition of TNF-α	[165]
Pentamidine	Inhibition of cytokine release	[166]
Surfactant	Inhibition of cytokine release Binding to endotoxin and *E. coli*	[167]
Taurolin	Inhibition of cytokine release	[168]
Thalidomide	Selective blocking of TNF-α release	[169]
Vitamin D_3	Inhibition of thromboxane A_2? Scavenging of free radicals?	[170]

NOTE. TNF = tumor necrosis factor.
† A coumarin derivative.

therapeutic agents. This approach has already yielded impressive dividends in clinical oncology and more recently in the developmental therapeutics of AIDS. Few animal studies and no human trials have addressed this strategy with regard to sepsis. The combination of a specific MAb to *Pseudomonas* with polyclonal antiserum to *E. coli* J5 and a neutralizing MAb to TNF-α was found to be more effective than single or double antibody treatment in a murine model of pseudomonas sepsis [171]. MAbs to IFN-γ and TNF-α provided greater protection than either antibody alone in a murine model [82]. However, no synergy between an MAb to IL-1 receptor and an MAb to TNF-α was evident in another murine model [75]. Further studies are required to define the most effective combination therapy.

Clearly, the assessment of these treatment regimens will be challenging, given the heterogeneous factors involved in sepsis and the difficulties encountered in clinical trials. The development of diagnostic tests that rapidly identify infecting organisms and the characterization of groups of patients likely to respond to specific types of adjunctive therapy would be of great value in the selection of appropriate treatment. If these goals can be accomplished, then we may finally see a reduction in the morbidity and mortality associated with this condition.

Note Added in Proof

Since this paper was prepared, several important studies have been reported. A recombinant 21 kD C-terminal fragment of BPI ($rBPI_{21}$) has been reported to inhibit imflammatory responses to endotoxin infusion in healthy volunteers [172]. The results of the INTERSEPT placebo-controlled trial of an MAb (Bay × 1351) to TNF-α in patients with severe sepsis have been reported [173]. In this trial 563 patients were randomized to receive placebo, 3 mg/kg of MAb, or 15 mg/kg of MAb. Mortality was not significantly altered between the groups: mortality among patients in the placebo group was 42.9%, among those in the 3 mg/kg group was 36.7%, and among those in the 15 mg/kg group was 44.6%. However, the study was not powered to detect a difference in mortality. Among the subset ot 247 patients who survived for 28 days, there was significantly more rapid reversal of shock in both treatment groups, supporting a beneficial action of the MAb. Metalloproteinase inhibitors have been shown to inhibit TNF-α processing and to protect rats from endotoxin challenge [174]. The results of the Immunex study of the p75 sTNFR:Fc have now been reported, and, as discussed above, there was an increase in mortality among patients in the treatment group [175]. A second phase 3 study of IL-1ra was halted after an interim analysis failed to show evidence of benefit. A PAF antagonist, TCV-309, has been shown to inhibit cytokine production in experimental endotoxemia in chimpanzees [176].

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