

Gram-Negative Sepsis: a Dilemma of Modern Medicine

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INTRODUCTION

Gram-negative sepsis, a relatively rare clinical diagnosis only a few decades ago, is perhaps the most important infectious disease problem in hospitals today. Despite recent advances in our understanding of the pathophysiological mechanisms of sepsis and improved antimicrobial therapy, the mortality rate from gram-negative sepsis remains frustratingly high, particularly after the onset of shock.

Unfortunately, many of the therapeutic methods proposed over the years for the management of sepsis and its complications have either failed to meet their initial expectations or remain unproved, despite many anecdotal reports. Recently, however, the development of new monoclonal antibody-based treatments, together with earlier recognition of and intervention in the pathogenetic process, has raised the hope that a significant reduction in deaths from gram-negative sepsis can be achieved.

This article reviews the epidemiology, diagnosis, and current management of gram-negative sepsis and examines the therapeutic potentials of new treatment modalities being developed.

DEFINITIONS

The American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (12) was the latest in a series of ongoing attempts (7-10) to provide a conceptual and practical framework in which to define the systemic inflammatory response to infection that often underlies sepsis. The term sepsis has traditionally been used to describe this progressive process, which is also associated with organ damage. Acceptance of the broad definitions proposed at the consensus conference would make early detection and treatment of disease possible and would facilitate the standardization of research protocols. The interpretation of clinical trials designed to evaluate conventional and innovative therapies for sepsis can be expected to improve if the use of disparate definitions for such terms as infection, bacteremia, sepsis, septicemia, sepsis syndrome, and septic shock can be avoided. The new terms and definitions proposed by the conference can be found in Table 1.

INCIDENCE AND NATURAL HISTORY

Sepsis is not a reportable disease, and it is possible that many deaths due to sepsis are attributed to underlying diseases when mortality statistics are compiled (86). Published estimates of up to 300,000 cases of sepsis per year in the United States may be realistic (47, 86). Estimated mortality from sepsis of gram-negative etiology ranges from 20 to 50% of the overall total number of septic deaths (75, 86); the fraction is notably higher among the approximately 40% of septic patients who develop shock. Among patients who develop the complications of shock and organ failure, mortality can reach 90% (9). Sepsis therefore represents a leading cause of death in the United States, and its incidence has increased significantly over the past decade (20).

A significant proportion of sepsis cases are caused by gram-negative bacilli (19). Table 2 shows the distribution of gram-negative isolates and their associated mortality rates, as summarized by Young from 11 studies reported from 1955 to 1986 (86). In that review, *Escherichia coli* was the most commonly isolated pathogen, followed by *Klebsiella* and *Enterobacter* species (86). Although *Pseudomonas* species were encountered somewhat less frequently, *Pseudomonas aeruginosa* has consistently been associated with the highest mortality rate among all causes of bacteremic infection (86). In the studies listed in Table 2, the mortality rates associated with gram-negative sepsis were as high as 61% and exceeded 25% in all but three of the centers involved.

From 1987 to 1988, a number of centers participated in a prospective national study of the natural history of gram-negative sepsis (49). A total of 226 patients with presumed gram-negative sepsis were available for analysis. Gram-negative bacteria were isolated from 152 patients (67%). At day 14, mortality was 26% for those patients with documented gram-negative sepsis and 23% for those from whom no gram-negative organism was isolated. The presence of the adult respiratory distress syndrome or disseminated intravascular coagulation during the first week of illness was the variable most predictive of death.

RISK FACTORS

Factors considered important in the development of gram-negative sepsis include broad-spectrum antibiotic therapy, immunosuppressive treatments (cancer chemotherapy, radi-

TABLE 1. Definition of terms used^a

Term	Definition
Infection	Microbial phenomenon characterized by an inflammatory response to the presence of microorganisms or the invasion of normally sterile host tissue by those organisms
Bacteremia	Presence of viable bacteria in blood
SIRS	Systemic inflammatory response to a variety of severe clinical insults (see text). The response is manifested by two or more of the following conditions: temperature, >38 or <36°C; heart rate, >90 beats per min; respiratory rate, >20 breaths per min, or PaCO ₂ , <32 mm Hg; leukocyte count, >12,000 cells per ml ³ , <4,000 cells per ml ³ , or >10% immature (band) forms.
Sepsis	Systemic response to infection. This systemic response is manifested by two or more of the following conditions as a result of infection: temperature, >38 or <36°C; heart rate, >90 beats per min; respiratory rate, >20 breaths per min, or PaCO ₂ , <32 mm Hg; leukocyte count, >12,000 cells per ml ³ , <4,000 cells per ml ³ , or >10% immature (band) forms.
Severe sepsis	Sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status.
Septic shock	Sepsis with hypotension despite adequate fluid resuscitation, along with the presence of perfusion abnormalities which may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status. Patients who are on inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured.
Hypotension	Systolic blood pressure of <90 mm Hg or a reduction of >40 mm Hg from baseline in the absence of other causes of hypotension
Multiple organ dysfunction syndrome	Presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention

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ation therapy, agents to reduce transplant rejection, and steroids), invasive devices or procedures (surgery, vascular and bladder catheters, prosthetic devices, drainage tubes, and inhalation therapy equipment), penetrating wounds,

burns or other trauma, anatomic obstruction, intestinal ulceration, age (the very young and the very old), and progressive clinical conditions (malignancy, diabetes, AIDS, and other serious chronic diseases) (14, 50, 53, 83). These forms of treatment may either serve as a source or a focus of infection that foments sepsis or they may prolong the life of a critically ill patient who is vulnerable to the disorder.

Contaminated intravenous fluids have been implicated in a number of nationwide epidemics of bacteremia. Between 1965 and 1978, Maki recorded 33 epidemics traceable to some form of infusion therapy; 7 of these were related to a contaminated commercial product (48). Nearly 80% of all documented epidemics were caused by gram-negative bacilli.

Although the survival of patients with progressive or chronic illnesses has been prolonged by better treatments for the primary disease, debilitation eventually occurs, and these patients become the immunocompromised targets of systemic infection.

DIAGNOSIS

In recent studies on the efficacy of methylprednisolone treatment for septic patients (13, 14), the inclusion criteria for study subjects included the following: a presumed site of infection, hyper- or hypothermia, tachycardia, tachypnea, and inadequate organ perfusion or function. Manifestations of insufficient perfusion included altered mental state, hypoxemia, elevated levels of plasma lactate, and oliguria. Bacteremia and hypotension were not essential for the diagnosis of sepsis syndrome in those studies.

Fever, the most common sign of sepsis, is believed to be caused by the actions of a number of endogenous substances on prostaglandin E₂ synthesis (3, 25, 26, 37). Hypothermia is seen principally in older patients (33, 37). Cardiac manifestations of sepsis range from tachycardia and increased cardiac output to myocardial failure (37). Respiratory signs of sepsis include respiratory alkalosis, hyperventilation, failure of respiratory muscles, and the adult respiratory distress syndrome, considered a catastrophic complication (6, 42, 54, 86).

An increase in cardiac output is often seen early in the course of the systemic inflammatory response syndrome (SIRS) but is usually offset by decreased peripheral resistance in the preshock state (37). Early shock is accompanied by a significant decline in systemic vascular resistance that may precede the fall in blood pressure (37). In later shock, declining cardiac output, vasoconstriction, and refractory hypotension may occur; alternatively, vasodilation may persist even in late shock (37, 86).

Renal manifestations of SIRS include azotemia and oliguria that result from renal tubular injury (37). Liver dysfunction may be revealed by a rise in serum bilirubin levels that frequently precedes the clinical signs of infection (29). Hematological abnormalities associated with SIRS include eosinopenia (37), vacuolization of neutrophils (91), reduced levels of iron in the serum (41), and the disseminated intravascular coagulation syndrome (37). Thrombocytopenia is often noted at an early stage of SIRS (58), as is hyperglycemia in diabetic patients (36, 37). A variety of changes in mental status is possible in the septic patient, including disorientation, lethargy, confusion, agitation, and obtundation (37, 86).

TABLE 2. Distribution of gram-negative bacteremic isolates excluding polymicrobial infections^a

Period of observation	No. of isolates (% mortality)						Total episodes (% mortality)
	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Enterobacter</i> spp.	<i>Serratia</i> spp.	<i>P. aeruginosa</i>	<i>Proteus</i> spp.	
1983-1986	63	9			34		129 (12)
1981-1983	38	8			23		83 (17)
1975-1977	76 (35)	25 (48)			22 (72)		123 (39.8)
1968-1974	127 (38.6)	233 (31.8)	67 (35.8)	37 (32.4)	74 (68.9) ^b	30 (36.7)	568 (38.9)
1965-1974	189 (19.5)	74 (24.3)	47 (17.0)	11 (18.0)	60 (36.6)	49 (16.3)	430 (22.1)
1972-1973	86 (17.4)	37 (43.2)		4 (0)	30 (60)	14 (35.7)	171 (31.6)
1967-1972	68 (26.4)	72 (26.9)	35 (14.3)	8 (25)	29 (48.3)	11 (27.3)	233 (27.3)
1968-1969	58 (18)	23 (13)	9 (0)	27 (54)	21 (57) ^b	13 (16)	151 (27.8)
1965-1968	83 (21)	57 (33) ^b			45 (71)	19 (16)	204 (34.8)
1958-1966	190 (42)	138 (55) ^b			67 (67) ^b	63 (33)	458 (50.7)
1955-1967	93 (48)	68 (66) ^b			39 (77)	42 (67)	242 (61.2)
Usual rank order frequency	1	2	5 ^c	4 ^c	3	6 ^c	
Mortality	4 ^c	2	6 ^c	5 ^c	1	3 ^c	

^a Reprinted from reference 86 with the permission of the publisher.

^b Species are grouped.

^c No significant differences in rank order.

PATHOGENESIS

At the pathophysiological level, the development of gram-negative sepsis involves a complicated series of effects based on the composition of the bacterial cell wall. Pfeiffer first recognized the heat-stable toxic component of gram-negative bacteria at the end of the 19th century (15, 56). In his experiments, Pfeiffer noted that lysates of heat-inactivated *Vibrio cholerae* caused shock and death in laboratory animals. He called the toxic substance, not yet characterized, "endotoxin" on the assumption that it was found inside the bacterium. This also served to distinguish it from toxins secreted during bacterial growth in culture (51, 56).

In the 1930s, endotoxins were isolated and characterized as lipopolysaccharide (LPS)-phospholipid-protein complexes present in the bacterial outer membrane (8, 51). Subsequent efforts yielded purified and protein-free LPS that could produce all of the physiological effects of the impure substance isolated earlier (51, 78). Later experiments suggested that a chemical subunit of LPS, lipid A, was the actually toxic moiety (51, 77) and that the O-specific chain found on LPS was not involved in the toxic effect (15, 31).

The structure of the LPS molecule is shown schematically

in Fig. 1 (27, 59). LPS is largely made up of a long-chain polysaccharide (O antigen), the core, and lipid A. Each of these regions is immunogenic. The O antigen shows great diversity of structure among the various strains of gram-negative bacteria. Thus, it has a great number of epitopes and considerable potential for antigenic activity (27, 30). On the other hand, lipid A is the most highly conserved subunit of the gram-negative LPS structure (51). Chemically, the toxic lipid A moiety has been characterized as an ester-linked glucosamine with both ester- and amide-linked pyrophosphates and fatty acids (17, 51). The form of lipid A produced by *E. coli* has now been synthesized in the laboratory (15, 39).

The physiological effects of endotoxin in vivo and the biochemical mechanisms underlying these effects have been extensively investigated. The administration of small doses of endotoxin to animals affects their hemodynamics, body temperature, blood clotting, cellular and humoral immunities, and other important physiologic parameters; large doses are lethal (16). In most species, the injection of LPS is associated with a rapid onset of fever, hypotension, and neutropenia (16). In rabbits injected with LPS, body temper-

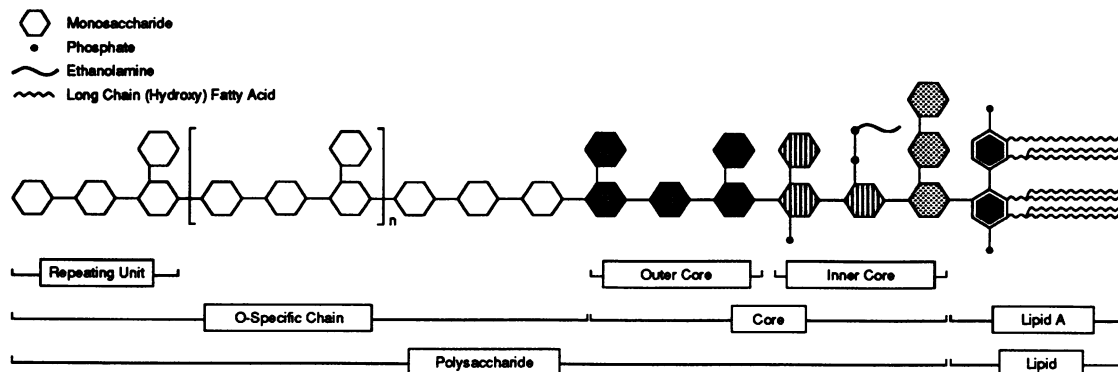


FIG. 1. Schematic representation of an enterobacterial LPS. Reprinted from reference 60 with the permission of the publisher.

ature begins to rise in 10 to 20 min and peaks at approximately 70 min (16). Humans are more sensitive to the pyrogenic activity of LPS and demonstrate fever at a small fraction of the LPS dosage required to cause the febrile response in rabbits (82).

Marked hypotension is observed in most species about 30 min after the administration of LPS (16). Recently, Suffredini et al. characterized the hemodynamic effects of endotoxin in humans by administering purified LPS to healthy volunteers (68). Three hours after dosing, systemic vascular resistance and mean arterial pressure had decreased by 46 and 18%, respectively, while the cardiac index had increased by 53% and the heart rate had increased by 36%. Left ventricular function, both before and after volume loading, was consistent with the hemodynamic alterations observed in septic shock.

The profound effects of endotoxin on clotting are demonstrated by both local and generalized Schwartzman reactions (65). In animal studies, these reactions have been incited by two injections of endotoxin 12 to 18 h apart. In the local reaction, an intradermal injection followed by an intravenous injection produces hemorrhagic necrosis at the extradermal injection site. In the generalized reaction, sequential intravenous injections produce bilateral renal cortical necrosis in the test animals. This occurs as a result of the occlusion of small vessels by fibrin and intravascular coagulation (16).

Endotoxin can also affect the blood cells, inducing neutropenia, leukocytosis, and a reduction in circulating platelets (16). The proliferation of B lymphocytes and macrophages is also stimulated by endotoxin.

It is generally agreed that most of the adverse effects associated with endotoxin result from its capacity to cause the release of various endogenous mediators and to act on a number of important biochemical pathways, as shown in Fig. 2 (53). The cytokines are an important group of mediators whose release occurs in sepsis. These include tumor necrosis factor and interleukin-1, both released by macrophages (86). Tumor necrosis factor is believed to be a primary mediator of the events that occur in sepsis, since the direct infusion of a recombinant form of this mediator produces most of the adverse effects seen after endotoxin administration (69).

Tumor necrosis factor and interleukin-1 are both endogenous pyrogens that contribute to the febrile response seen in sepsis (27, 86). Tumor necrosis factor may also act synergistically with interleukin-1, gamma interferon, or both to trigger a systemic inflammatory response and cause damage to the vascular endothelium (86). Tumor necrosis factor may also provoke the release of prostaglandins (86) and other lipid mediators of shock, including platelet-activating factor, leukotrienes C₄ and D₄, and thromboxane A₂ (44). The adverse effects of these lipid mediators include increased vascular permeability and vasoactivity and the contraction of pulmonary smooth muscle (16, 44).

Another key action of endotoxin is its effect on the coagulation system. Endotoxin activates factor XII (Hageman factor), which in turn initiates the intrinsic clotting sequence that eventually results in the conversion of fibrinogen (factor 1) to fibrin (86). The continued activity of endotoxin, especially in the presence of shock, can lead to thrombosis and the excessive consumption of platelets and coagulation factors II, V, and VII (86). The clinical expression used to describe this series of effects is coagulopathy, or disseminated intravascular coagulation. The activation of Hageman factor by endotoxin is also an initial step in

complement and kinin system activation. Complement activation by endotoxin can take place by way of both the classic and the alternative pathways (16, 86).

Although the complement system is important in the lysis and phagocytosis of pathogenic organisms, overstimulation of the system can have deleterious effects. One such event is the increased chemotaxis of polymorphonuclear leukocytes caused by complement activation, which can produce pulmonary leukostasis, an important factor in the development of the adult respiratory distress syndrome (63).

After its activation by endotoxin, Hageman factor also stimulates the conversion of prekallikrein to kallikrein and the subsequent conversion of kininogen to bradykinin (86). Bradykinin can have a number of adverse effects on the vascular system, including an increase in vascular permeability and a decrease in vascular resistance that can lead to hypotension (52, 86).

Other endogenous vasoactive substances that are probably affected by endotoxin are catecholamines, endorphins, the neurotransmitter serotonin, and adrenal corticoids (86). The mechanisms by which these mediators are released and the clinical significance of their release are subjects for further investigation.

Antibodies of the immunoglobulin G (IgG) and IgM classes that are directed against O and K polysaccharide antigens have opsonic and bactericidal activities, especially in the presence of complement (86). Other antibodies directed against the core regions of the gram-negative bacterial cell wall appear to neutralize endotoxin (86). These observations provide the rationale for efforts to develop an anti-endotoxin antiserum with broad reactivity against the cell walls of important gram-negative pathogens. Advances along this line of research along with the effective use of hybridoma technology have produced new agents with exciting potential to fight sepsis. Some of these agents are being tested and should soon be commercially available. These developments are discussed in greater detail in the final section of this review.

MANAGEMENT

The early administration of appropriate antimicrobial therapy is an important aspect of the effective management of sepsis (70, 86). In one large study, treatment with appropriate antibiotics reduced shock and mortality rates by 50% (43). Because the results of blood culture and susceptibility testing cannot usually be provided in less than 48 to 72 h and because more than 50% of the deaths caused by gram-negative sepsis occur during the first 2 days of the illness, empirical, parenteral, broad-spectrum antibacterial therapy is a widely accepted treatment mode (43, 70, 86).

Therapy for presumed gram-negative sepsis usually consists of a combination of agents. For instance, an aminoglycoside plus either expanded-spectrum cephalosporin or an antipseudomonal penicillin may be used. Treatment can be tailored to specific pathogens when culture and susceptibility results are available. Drug selection should be based on several factors, including the site or presumed site of infection, the place from which the infection was acquired (community or hospital), the underlying disease status, possible drug toxicities, and the likelihood of drug resistance (70).

Although the most frequently used aminoglycosides are gentamicin and tobramycin, an agent such as amikacin or netilmicin may be substituted if resistance to the primary drugs is likely on the basis of institutional experience or the patient's risk status (86). Peak levels of gentamicin or

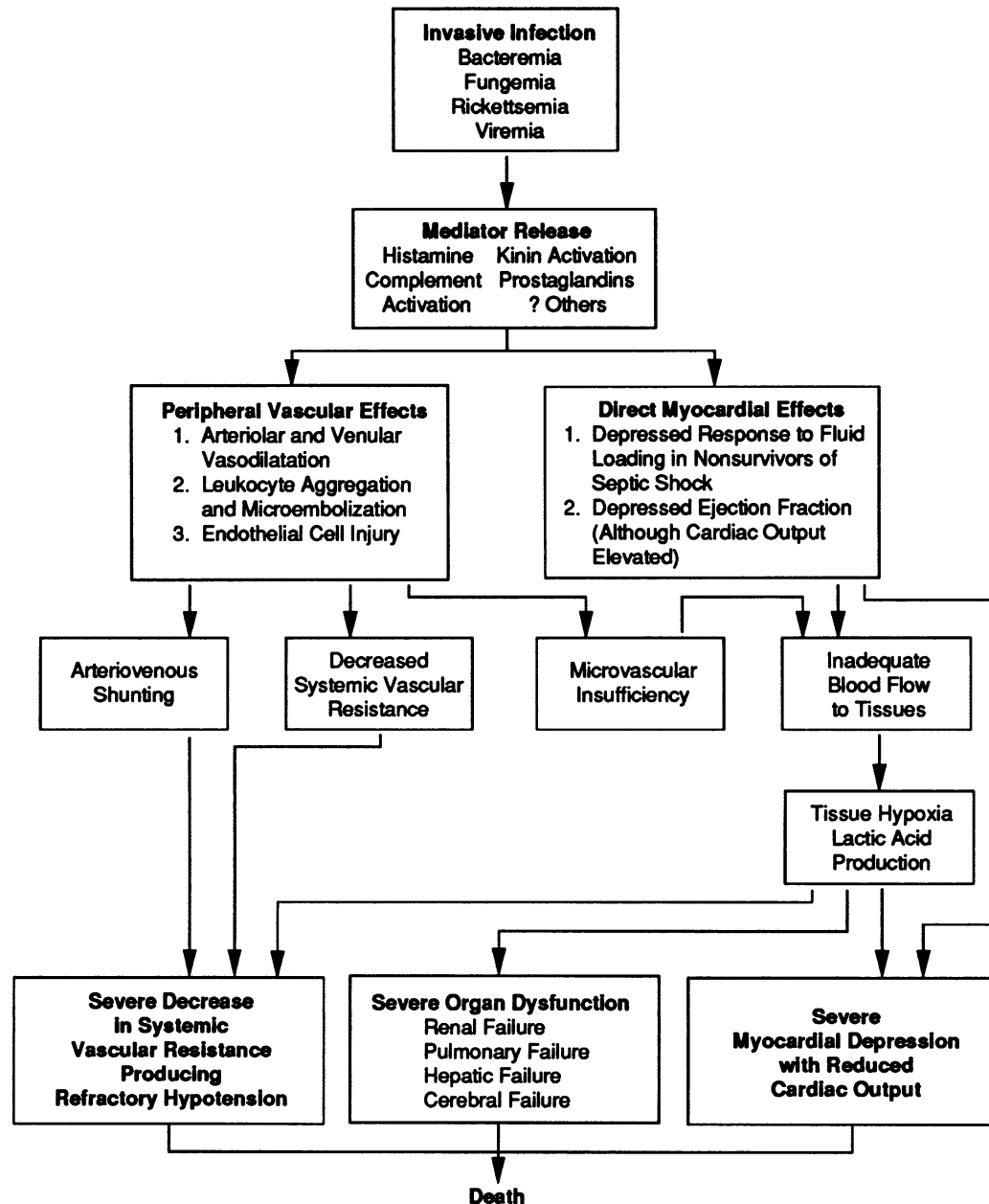


FIG. 2. The septic cascade: pathogenesis of septic shock. Reprinted from reference 53 with the permission of the publisher.

tobramycin in the blood should be maintained at between 6 and 10 $\mu\text{g}/\text{dl}$, while trough levels should fall below 2 $\mu\text{g}/\text{dl}$ to decrease the chances of ototoxicity or nephrotoxicity (62). Frequent monitoring of drug levels in the blood is therefore required.

For hospital-acquired infections in non-neutropenic patients, an expanded-spectrum cephalosporin rather than an aminoglycoside is often used because the etiologic organism is more likely to be a *Klebsiella* sp. than a *Pseudomonas* sp. In patients with presumed *P. aeruginosa* infection, including those with neutropenia, burns, or infection related to respiratory therapy, an antipseudomonal penicillin such as mezlocillin, piperacillin, ticarcillin, or azlocillin may be substituted for the cephalosporin and used in combination with an aminoglycoside (85). In the case of resistance to both cep-

alosporins and penicillins, imipenem may be used with the aminoglycoside (86).

The rationale for using a combination of two antibiotics is based on several considerations, including the broad coverage of potential pathogens, the frequency of polymicrobial infections, and the possibility of antibacterial synergy between the two agents. Such synergistic combinations have been associated with improved clinical results (2). In addition, such combinations may reduce the chances of emergent resistance by eliminating secondary bacterial populations that are resistant to one drug but not both (86). Current evidence does not support the use of triple-drug combinations, and combinations of bactericidal and bacteriostatic agents should generally be avoided (40).

The appropriate management of fluid and electrolyte bal-

TABLE 3. Sympathomimetic amines for circulatory support in septic shock^a

Drug	Dose ($\mu\text{g}/\text{kg}/\text{min}$)	Comments
Dopamine	2-25	Increase infusion rate (D_5W or saline) every 15-20 min until systolic blood pressure exceeds 90 mm Hg and urine output exceeds 30 ml/h.
Dobutamine	2-25	Titrate as for dopamine.
Isoproterenol	5	Observe effect within 15-25 min; double infusion rate if necessary.
Norepinephrine	0.05	Start with test dose of 0.1-0.2 $\mu\text{g}/\text{kg}$ and observe within 5 min; administer via plastic catheter into large peripheral or central vein.

^a Adapted from reference 86 with the permission of the publisher. Drugs are listed in order of preference and are to be used after volume replacement and with careful electrocardiograph, central venous pressure, and blood pressure monitoring. D_5W , 5% dextrose in water.

ance is an important supportive measure in the treatment of sepsis, particularly when shock ensues. Sympathomimetic amines may also be administered to manage the hemodynamic complications encountered in septic shock. Table 3 summarizes the recommended sympathomimetic amines for use to control shock. Dopamine raises the heart rate and systolic blood pressure at higher infusion rates. Many clinicians prefer to use low-dose dopamine (1 to 10 $\mu\text{g}/\text{kg}$ of body weight per min) for its effect on renal perfusion (dopaminergic effect). Dobutamine may be added to the therapeutic regimen to increase myocardial contractility. If systolic blood pressure is still not adequate, norepinephrine is titrated to increase blood pressure through an increase in systemic vascular resistance. Compared with dopamine, dobutamine has less influence on heart rate and causes a decrease in pulmonary capillary wedge pressure. Isoproterenol does not markedly elevate blood pressure, but it does increase the cardiac index (79). Adequate volume replacement must be achieved before any sympathomimetic amine is administered (86).

Despite extensive investigation, the utility of a number of drugs in the treatment of septic shock remains controversial. Disagreement concerning the use of glucocorticoids has persisted for many years. The finding that corticosteroid treatment improved survival in laboratory animal models of sepsis was supported by the results of a 1976 clinical trial (64). Although this study was prospective and randomized, concerns were raised regarding certain aspects of the trial design. Sprung et al. compared the effect of a two-dose steroid regimen with that of placebo in patients with septic shock and found no significant between-treatment differences in mortality rates (66). In 1987, two large, controlled trials of glucocorticoid therapy in sepsis were published simultaneously, one by The Veterans Administration Systemic Sepsis Cooperative Study Group (72) and the other by the Methylprednisolone Severe Sepsis Study Group (13). Both trials were prospective, randomized comparisons of high-dose methylprednisolone sodium succinate and placebo. In the Veterans Administration study, 14-day mortal-

ity was similar in the glucocorticoid (21%) and placebo (22%) groups. The resolution of secondary infection was significantly higher in patients who received placebo ($P = 0.03$).

In the second study of methylprednisolone, mortality at 14 days was not improved by steroids, nor were treatment-related differences in the reversal of shock observed. The authors of both of these important studies concluded that high-dose glucocorticoid therapy provides no benefit in patients with sepsis and septic shock and should not be used. It is now widely accepted that glucocorticoids should not be used in the treatment of septic shock (57).

The opiate antagonist naloxone has attracted interest because of its capacity to alter endotoxic shock in animals (28). In a small trial published in 1981, Peters et al. observed a 45% increase in systolic blood pressure after the administration of 0.4 to 1.2 mg of naloxone to eight patients with sepsis who were not receiving corticosteroids (55). Increased blood pressure was evident within a few minutes of the intravenous injection and lasted for about 45 min. Four years later, however, DeMaria and associates performed a double-blind, placebo-controlled study of intravenous bolus naloxone in septic shock patients and found no significant between-treatment differences in either blood pressure elevation or survival (24). In the most recent study of naloxone in the treatment of sepsis, Roberts et al. gave either placebo or a 30- $\mu\text{g}/\text{kg}$ intravenous bolus injection plus an additional 30- $\mu\text{g}/\text{kg}$ infusion of naloxone to 14 patients with septic shock who required the support of inotropes, vasopressors, or both (61). The infusions of naloxone or placebo were administered over a period of 16 to 18 h. Pulmonary wedge pressure and pH were kept constant, and inotrope or vasopressor therapy was titrated to maintain a fixed mean blood pressure. Inotrope or vasopressor requirements were significantly lower in the naloxone-treated group than in the placebo group at 8 ($P < 0.005$) and 16 ($P < 0.02$) h. Significant improvements were also seen in stroke volume and heart rate in the group that received naloxone compared with those who received placebo. Because the positive hemodynamic effects seen in the naloxone group were observed only after 4 h, earlier studies utilizing bolus injections may not have provided an optimal drug regimen and observation period. Additional studies with naloxone are needed to clarify its role in the management of septic shock.

Anticoagulants, particularly heparin, have been widely used in the management of disseminated intravascular coagulation. Although these agents can ameliorate the clinical expressions of coagulopathy (23), they have not been shown to reduce mortality, and their use is probably best reserved for other indications (86).

Transfusions of granulocytes for both the prophylaxis and treatment of sepsis have been studied. In trials involving prophylactic transfusions, investigators augmented the circulating granulocyte pool in patients scheduled to undergo aggressive chemotherapy for bone marrow transplantation or leukemia (22, 67, 81). The results of these studies suggested that any modest reductions in the incidence of gram-negative infection due to the infusions were countered by an increased incidence of pulmonary problems. This prophylactic strategy cannot be recommended.

Therapeutic granulocyte transfusions have yielded more encouraging results than have prophylactic transfusions; however, the evidence to date does not support the use of granulocytes in the routine treatment of neutropenic patients. A survival benefit noted in some small studies (1, 38) was not confirmed by a larger, randomized study by Winston et al. (80). In this large trial, granulocytopenic patients

TABLE 4. Dependence of mortality rate on presence or absence of shock at study admission^a

Study group	No. of patients with symptoms/total no. of patients (% affected)		
	Only sepsis syndrome present	Shock at admission	Shock developed after admission
Total ^b	10/77 (13)	19/69 (28) ^c	19/44 (43) ^d
Nonbacteremic	8/50 (16)	7/34 (21)	9/20 (45) ^d
Bacteremic	2/26 (8)	11/34 ^e (32)	10/24 (42) ^f
Gram-negative	1/16 (6)	8/23 (35)	7/16 (43) ^c
Gram-positive	1/10	3/11 (27)	3/8 (38)

^a Reprinted from reference 14 with the permission of the publisher.

^b Follow-up data were available for 190 of 191 patients (mortality data were not available for one patient in the sepsis-syndrome-only group).

^c $P < 0.05$ compared with the sepsis-syndrome-only group.

^d $P < 0.01$ compared with the sepsis-syndrome-only group.

^e One patient with shock present on admission died from a primary fungemia.

^f $P < 0.001$ compared with the sepsis-syndrome-only group.

(granulocyte count of $<0.5 \times 10^9$) with proven infections were randomly assigned to receive either a daily granulocyte transfusion or a control treatment. In addition to this treatment, antimicrobial therapy was also instituted. Among patients with gram-negative sepsis who did not demonstrate bone marrow recovery, the survival rate was 48% in the transfused group and 50% in the control group. The authors concluded that granulocyte transfusion conferred no significant benefit over that of optimal antimicrobial therapy alone.

It has been suggested that the therapeutic use of granulocytes might prove more efficacious if larger quantities of cells could be administered (85). While currently unfeasible, improved techniques in the harvest of granulocytes could someday make this possible. In the meantime, these transfusions should be reserved for patients with reversibly defective granulocyte production who have not responded to appropriate antimicrobial therapy.

The prognosis for patients with sepsis becomes significantly more grave at the onset of septic shock. In our own prospective study (Table 4), patients with sepsis and without shock had a mortality rate of 13% (13). The mortality rate was 28% for septic patients with shock at trial entry and 43% for those who developed shock after entry. These values highlight a need for the aggressive treatment of sepsis at the earliest possible time during its course. This has been identified as a means of preventing shock and improving outcome. These efforts should be helped considerably by the therapeutic modalities now being developed.

ADVANCES IN THE MANAGEMENT OF SEPSIS

Immunotherapy for infection was first practiced more than 100 years ago when von Behring used an equine antiserum to treat patients with diphtheria (21). The early years of this century saw the development of antisera against a number of important bacteria. The antibodies against a particular bacterium recognized and acted against that organism only, however (21). This line of research was almost entirely discontinued after the introduction of effective antimicrobial agents, but interest in serum therapy reemerged when it was realized that broad-spectrum agents were not having the expected impact on mortality caused by bacterial sepsis.

The promise of immunotherapy in the treatment of sepsis was underscored in 1982, when Ziegler and colleagues

published the results of their clinical study on a polyclonal antiserum against gram-negative bacteria (89). Earlier researchers had determined that, whereas the oligosaccharide side chains of gram-negative bacterial LPS differ widely from strain to strain, the core regions of most strains are quite similar (45). Thus, it was conjectured, broadly effective immunotherapy for gram-negative sepsis in the form of antibodies raised against LPS might be developed through the use of a bacterial strain with an outer membrane that features no side chains, instead bearing only the conserved core elements in its LPS. The strain selected was the J5 mutant of *E. coli* O111:B4, whose LPS contains only the core determinants, primarily lipid A.

After encouraging results were obtained in animal experiments, the researchers prepared human J5 antiserum by immunizing healthy donors with a J5 boiled-cell vaccine (91). Over a period of 7 years, 304 patients with clinical symptoms that suggested gram-negative bacteremia were entered into the trial and randomized to treatment with either 1 U of immune serum or the same quantity of preimmune serum as a control. Of the 212 patients in whom the diagnosis of gram-negative bacteremia was subsequently confirmed, 103 received immune serum and 109 received control serum. Mortality was significantly lower (22%) in the J5 antiserum group than in the control group (39%). In the subset of patients with profound shock who needed vasopressors for more than 6 h, mortality was 77% in the control group and 44% in the antiserum group. The authors concluded that J5 antiserum reduced mortality from gram-negative sepsis by approximately 50% and that this protection was apparent even in the presence of optimal antimicrobial therapy and medical-surgical management (91).

These findings led to a prophylactic trial of J5 antibody in which high-risk surgical patients were treated with either immune or preimmune plasma at the time of randomization and every 5 days thereafter until they were no longer considered to be at high risk (5). Because the previous therapeutic trial involving J5 had not shown a significant decrease in mortality among the patients with abdominal infections, the effect of prophylaxis in this group was of considerable interest. In addition, the study allowed researchers to assess the value of this strategy in patients at substantial risk, such as those with multiple trauma, the elderly, and immunocompromised patients undergoing lung surgery.

A total of 262 evaluable patients were entered into the study and observed daily. Although there was no significant difference in the incidence of infection in the J5 antibody study group (36%) compared with the control group (40%), the antibody clearly reduced the serious consequences of gram-negative infection. Patients in the control group had a risk of developing septic shock that was more than twice that in the antibody group. A twofold decrease in mortality was observed among patients in shock who received J5 plasma. The antibody's efficacy was also demonstrated in patients who underwent abdominal surgery. The study also confirmed the specificity of J5's affinity for lipid A, the injurious portion of the bacterial outer membrane, since neither the direct consequences of gram-positive infection nor the infection rate was altered, while the complications of gram-negative infection, such as the inflammatory response, were affected. This suggests that the J5 antibody bound directly to the lipid A portion of the bacteria (5).

In patients with SIRS, retrospective analysis had demonstrated a significant correlation between the serum titers of antibody to core glycolipid and survival (47). Nevertheless,

the relationship between the level of J5 antibody achieved in septic patients and the improvement seen in their outcome remained to be demonstrated (4, 91). Important follow-up studies indicated that a human IgG antibody to *E. coli* J5 was not effective in reducing complications of gram-negative sepsis (18) and that the protective activity of the antiserum lay almost entirely in the IgM antibody (46).

Despite these encouraging results, polyclonal J5 antiserum is not suitable for commercial development for a number of reasons. These include the adverse effects of vaccination on serum donors; the variability of antiserum activity; the need to use pooled human blood, thereby creating a risk for transmission of viral disease; and the difficulty of producing large quantities of antiserum.

Fortunately, the discovery and elaboration of hybridoma technology have allowed the mass production of IgM monoclonal antibodies that may prove to be useful in the management of gram-negative sepsis. Preclinical and clinical studies on both murine and human monoclonal antibodies have been encouraging. A detailed analysis of these monoclonal antibodies follows.

On the basis of the obvious promises held out by anti-endotoxin therapy, the anti-endotoxin antibody E5 was developed, using the following procedure (32). After mice were immunized with boiled *E. coli* J5 cells, their spleen cells were fused with murine myeloma cells to yield a single (monoclonal) cell line producing an antibody directed specifically against lipid A. Selection of the proper cell line provided a culture that continued to produce an IgM antibody that reacts with the core region of gram-negative bacterial outer membranes (71). A recent *in vitro* study that employed boiled bacterial cells and a large panel of LPS and lipid A preparations found that lipid A was the apparent epitope on LPS to which E5 binds (84).

The results seen in preclinical and clinical trials of E5 have been encouraging. After significantly improved survival was seen in mice challenged with gram-negative organisms and treated with E5 (87), and following human pharmacokinetic studies (74), two major clinical trials of E5 were undertaken. In the first trial, a double-blind comparison of E5 and placebo in patients with suspected gram-negative sepsis showed that mortality among those in the subgroup with documented gram-negative infection was 22% in patients given routine therapy alone but only 7% in patients given routine therapy plus E5 (34).

In a subsequent large, double-blind trial in which 33 centers participated, 486 patients with suspected gram-negative sepsis were enrolled (35). To be eligible, patients were required to show at least two of the following signs of gram-negative infection: a core temperature of >38 or $<35^{\circ}\text{C}$; a peripheral blood leukocyte count of $>12 \times 10^9$ or $<3 \times 10^9$ /liter (not resulting from other treatments), or $\geq 20\%$ immature forms; growth of gram-negative bacteria from a blood culture taken within the preceding 48 h; and documented or suspected site of gram-negative infection. In addition, evidence of a systemic response was required (Table 5). The presence of at least one of the following symptoms was taken as evidence of such a response: arterial hypotension; metabolic acidosis; decreased systemic vascular resistance; tachypnea; or otherwise unexplained dysfunctions of the kidney, central nervous system, lung, or coagulation systems.

Grounds for exclusion from the study included the occurrence of any one of the following: uncomplicated transient bacteremia; granulocyte count of $<10^9$ /liter, late septic shock, core temperature of $>41.7^{\circ}\text{C}$, Child's class C liver

TABLE 5. Evidence of systemic response in the E5 trial

Parameter	Response
Arterial hypotension	Systolic blood pressure of <90 mm Hg or acute drop of 30 mm Hg
Metabolic acidosis	Base deficit of >5 meq/liter
Decreased systemic vascular resistance	Resistance of <800 dynes/s/cm ⁵
Tachypnea	Respiratory rate of <30 breaths/min or mechanical ventilation of >10 liters/min
Unexplained dysfunction of	
Kidney	Urine output of <30 ml
Lung	PaO ₂ of <0.1 atm on room air or PaO ₂ /FIO ₂ of <0.37 atm ^a
Central nervous system	Confusion, disorientation, obtundation
Coagulation	Thrombocytopenia (25% decrease in platelet count), prolonged prothrombin, or partial thromboplastin times

^a PaO₂, oxygen tension of arterial blood; FIO₂, oxygen fraction of inspired air. 1 atm = 101.29 kPa.

disease, AIDS, burn infections, pregnancy or lactation, previous treatment with or a known allergy to murine products, treatment with any other investigational agent, age under 18 years, or lack of commitment to full life support by the primary physician.

Patients were randomly assigned to receive treatment with either placebo (D5NS) or 200 ml of a solution containing the E5 anti-endotoxin antibody at a dose of 2 mg/kg. The 1-h infusion was administered intravenously. A second infusion of the study drug was administered at the same dose 1 day later. Patients also continued to receive the antimicrobial agent and supportive therapy deemed appropriate by the primary physician. Because 18 patients were excluded from efficacy analysis, 468 were considered evaluable. Of these, 316 had documented gram-negative sepsis, as demonstrated by a positive culture result for any normally sterile body site during the period of 2 days before to 3 days after the administration of the study drug.

E5 was not significantly more effective than placebo in improving survival among the 316 patients with documented gram-negative sepsis. In the subgroup ($n = 137$) of patients with gram-negative sepsis who were not in refractory shock at study entry, however, patients taking E5 had a significantly lower mortality rate (hazard ratio = 2.3; $P = 0.01$). Both bacteremic and nonbacteremic patients contributed to improved survival in the E5 group as a whole, since they were associated with relative risks of 2.3 and 2.1, respectively. Survival data are summarized in Fig. 3.

Among the 137 patients with sepsis who were not in shock, the number of times that individual organ failures (disseminated intravascular coagulation, adult respiratory distress syndrome, or acute renal failure) resolved approached significance ($P = 0.05$) among patients taking E5 (19 of 35 resolved [54%]) compared with those taking placebo (8 of 27 resolved [30%]).

The two E5 studies have shown that the drug is well tolerated. Adverse reactions were seen in 5.9% of the E5 group, although these were not necessarily related to E5 administration, and in 2.1% of the control group. Rash and an anaphylactoid reaction were the most common adverse events probably related to E5 administration, with a frequency of 1.2 and 1.0%, respectively. The presence of a

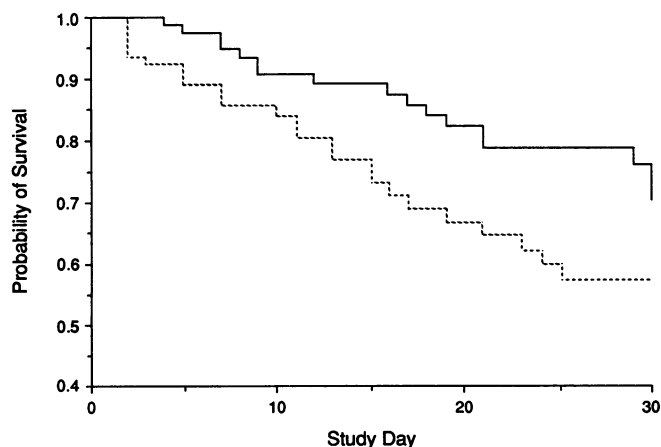


FIG. 3. Thirty-day Kaplan-Meier survival curves of patients with gram-negative sepsis. Solid line represents survival probability in the E5 group; dotted line represents survival probability in the placebo group. Reprinted from reference 35 with the permission of the publisher.

human antimurine antibody response was evaluated by an enzyme immunoassay method with a sensitivity of <1.0 ng/ml. A fourfold increase over baseline was considered a positive result. Serial determinations of human antimurine antibody response among 182 patients treated with E5 showed positive IgG responses in 86 patients (47%). Antibody responses generally occurred 2 weeks after the initial dose, however, and were not associated with clinical adverse events.

Encouraging results have also been reported with a human hybrid monoclonal antibody, HA-1A, derived from *E. coli* J5 vaccine (88). HA-1A was evaluated in 543 patients with sepsis syndrome and presumed gram-negative infection. The therapeutic regimen consisted of 100 mg of HA-1A or placebo (human serum albumin) administered in a single intravenous infusion. Patients were monitored for 28 days or until death. HA-1A was significantly more effective ($P = 0.014$) than placebo at reducing mortality in the subset of patients with documented gram-negative bacteremia ($n = 200$). HA-1A also reduced mortality by 42% in the patients with both bacteremia and shock ($P = 0.017$ compared with placebo). HA-1A did not offer significant protection to all patients entered into the study, however; treatment was not significantly protective in nonbacteremic patients.

During the first 7 days after treatment with HA-1A, all evidence of any major complications of sepsis present at baseline (such as shock, disseminated intravascular coagulation, acute renal failure, acute hepatic failure, or adult respiratory distress syndrome) resolved in 26 of 62 patients (42%) given placebo and in 38 of 61 (62%; $P = 0.024$) given HA-1A.

The use of HA-1A, too, was well tolerated by patients. One patient developed hives at the infusion site, and another experienced flushing and mild hypotension. These events were mild and transient in both patients. No patient had detectable anti-HA-1A antibodies.

The results of these studies indicate that both the murine and the human hybrid monoclonal anti-endotoxin antibodies improve survival in patients with gram-negative sepsis. E5 appears to be effective whether or not the patient is bacteremic, while HA-1A appears to be effective only in the presence of bacteremia. On this note, it is unfortunate that

the confirmation of bacteremia frequently occurs after the optimum time for pharmacologic intervention, by which time the patient may already be showing signs of SIRS. Another difference between the two antibodies is that HA-1A was effective in patients with shock refractory to treatment with fluids or inotropes, while E5 was not. However, direct comparisons between the two studies are difficult because of their different methodologies. For instance, the definitions for shock used by the two studies were different: some 40 patients who were characterized as not being in shock in the E5 study may have met the criteria for shock in the other study. In addition, the different antibody responses seen in the two studies might be related to the detection limits of the assays used. Limited sampling and the use of a less sensitive assay probably decreased the likelihood that significant antibody responses could be detected in the HA-1A study. It is important to note that antibody responses to E5 appeared well after completion of the 2-day course of therapy and were not associated with clinical sequelae.

While the results of these trials into the use of J5, E5, and HA-1A on patients with sepsis appear to be supportive, not all writers have agreed. For instance, an editorial by Wenzel concluded that these studies failed to show an effectiveness that would yield an acceptable cost-benefit ratio (76). He further pointed out that various confounding factors were present in the studies that may have resulted in differences between the control and treatment groups. These include supplemental treatment with antimicrobial agents and the presence of varying levels of disease at entrance.

In an editorial for the Sounding Board section in the same issue of the *New England Journal of Medicine*, however, Warren et al. come to a different conclusion on the issue of HA-1A anti-endotoxin antibody use (73). First, they think there is a need for more clinical research into the effects of the HA-1A antibody with stress on the idea that these antibodies are intended for adjunctive use along with appropriate antimicrobial therapy. However, they believe that there is not enough in vitro evidence of HA-1A's ability to effectively bind endotoxin. They quoted in vitro and animal studies of the antibody that bring into doubt its ability to bind to endotoxin and decrease the host's systemic response. They also had familiar critiques on the differences between the control and treatment groups in the clinical studies.

These articles were rebutted by a letter from Ziegler and Smith (90) found in the same issue. They think that there has been much in vitro work in the last 6 years showing the specificity and strength of HA-1A binding to endotoxin. They also pointed out that animal studies cannot be applied to human use of the antibody because of the divergent responses of animals to endotoxin.

To summarize the much discussed results of the trials into E5 and HA-1A use in septic patients, it is easy to criticize any study because of imbalances between the placebo and treatment groups. These will inevitably occur by chance if enough baseline criteria are analyzed. However, in my opinion, these studies of anti-endotoxin antibodies have been rigorously planned and well executed. I believe that, ultimately, both murine and human monoclonal antibodies will be highly useful. If these agents become generally available and commonly used, they should herald a significant advance in the management of gram-negative sepsis.

It should also be remembered that imagination is the only limit to the types of new agents that may be used to fight gram-negative sepsis. Currently, several are being developed for use by researchers (11). While the use of antibodies to endotoxin may be an important adjunct to the treatment of

gram-negative sepsis, many other key molecular interactions involved in the systemic inflammatory response may be affected through the use of novel agents. For instance, antibodies that bind to the cytokines themselves could directly affect the endogenous mechanisms by which sepsis occurs. Monoclonal antibodies to exotoxins, phospholipase A₂, C5a (a complement fragment), adhesion molecules, and contact factors could also become important in the fight to control SIRS. Similarly, agents can be found that block the tumor necrosis factor, interleukin-1, platelet-activating factor, thromboxane A₂, or bradykinin receptors, thus diminishing the deleterious effects of these mediators. Agents which inhibit neutrophil activation could be effective in blocking the inflammatory response. These include pentoxifyline, adenosine, dapsone, antioxidants, heavy-metal chelators, oxygen radical scavengers, and protease inhibitors. Coagulopathy is an important and deleterious part of the inflammatory response. Because of the great complexity of coagulation, there are numerous points at which the response may be controlled. Some inhibitors of this process include antithrombin III, protein C, thrombomodulin, hirudin, α_1 -antitrypsin Pittsburgh, aprotinin, soybean trypsin inhibitor, and plasminogen activators. Other therapeutic measures that may prove helpful in SIRS include gut decontamination, antihistamines, naloxone, thyroid releasing hormone, glucagon, surfactant, extracorporeal membrane oxygenation, calcium channel blockers, growth factors, and growth hormone. I think that all such agents should be subjected to intense investigation and scrutiny, although it is also important that we not reject potentially important advances in therapy that may provide improvements in patient management.

CONCLUSION

Gram-negative sepsis remains a significant cause of morbidity and mortality in spite of the ongoing development of new antimicrobial agents. This may be because antimicrobial therapy fails to address the underlying pathogenetic mechanism involved in the systemic inflammatory response. It is the triggering of mediators by bacterial endotoxin that produces the symptoms of gram-negative sepsis. Monoclonal antibody technology has made possible the development of preparations that, theoretically, will bind to and neutralize endotoxin, thereby inactivating it.

The anti-endotoxin antibody E5 has been shown to improve survival and enhance the resolution of major morbidities in patients with gram-negative sepsis who are not in shock. Similarly, HA-1A, a human hybrid monoclonal antibody, enhanced the resolution of organ failures and improved survival in bacteremic patients with gram-negative sepsis. These encouraging clinical results suggest that the administration of these antibodies early in the course of gram-negative sepsis as part of a therapeutic regimen that also includes antibiotics and appropriate supportive care should significantly affect morbidity and mortality.

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