Therapeutic Intervention Strategies in Endotoxemia*

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ABSTRACT: Endotoxemia and sepsis are complex biologic events involving the interplay of numerous inflammatory mediators that can be both essential for a protective response as well as damaging to the patient. Strategies for managing these conditions have involved blockage of the effects of endotoxin, modulation of the proinflammatory response, and up-regulation of the antiinflammatory response. The numerous mediators involved, their multitude of effects, and their sometimes-conflicting roles have made research difficult. Proinflammatory responses have been shown to be essential for an efficient immune reaction. No agent has proven universally beneficial in the face of preexisting sepsis. This article discusses the researched areas for beneficial intervention as well as pharmacologic treatments.

The outcome of some models of existing endotoxemia and sepsis has been worsened by the vigorous blockage of proinflammatory cytokines. An appropriately vigorous inflammatory response to infectious challenge is essential for survival of the host. However, if unchecked, this response can lead to detrimental effects. Concurrent with the development of the proinflammatory mediator cascade, an antiinflammatory response is generated within the host to modulate the overall immune reaction to a suitable level.

BIOLOGICAL RESPONSE MODIFIERS
Lipopolysaccharide Neutralization

Lipopolysaccharide (LPS) is the most important bacterial factor in the pathophysiology of sepsis and its sequelae (Figure 1). Experimentally, LPS levels have been shown to be inversely correlated with survival. Neutralization of the toxic properties of LPS is central to halting the inflammatory cascade leading to sepsis. There are three aims essential to neutralization (Figure 2):

1. LPS must be removed from its in vivo sites of action.
2. Functional molecules must be inactivated.
3. Interaction with receptors on susceptible cells must be prevented.

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Most research has focused on the development of cross-reactive antibodies directed against the conserved core and lipid A regions of LPS. The conserved nature of lipid A across gram-negative bacteria provides an opportunity for control. Antibody binding to the lipid A portion of LPS is dependent on cleavage of the polysaccharide portion; therefore, these antibodies do not react with intact gram-negative bacteria.

Immunotherapy using monoclonal anticore antibodies seems to improve survival in selected patients with gram-negative sepsis. Inhibition of the production of tumor necrosis factor (TNF)-α has been demonstrated. E5, which is a murine IgM directed against *Escherichia coli* J5 lipid A, has shown the ability to reduce endotoxin levels experimentally. Mortality rates have not been affected in humans with preexisting sepsis given this antibody, although preemptive use did improve survival. HA-1A, which is derived from a murine–human IgM-secreting heteromyeloma, is a second monoclonal antibody used in trials. Overall survival was not improved with this antibody and in some cases worsened. Neither E5 nor HA-1A was shown to neutralize LPS, prevent LPS-induced B-cell proliferation, or inhibit LPS-induced cytokine secretion. Numerous other monoclonal antibodies have been investigated with varying success.

In one study of horses with acute toxic enteritis, volvulus of the large colon, proximal jejunitis/duodenitis, or strangulating obstruction of the small intestine, the use of J5 hyperimmune plasma containing polyclonal antibodies in addition to traditional therapy (e.g., fluids, antimicrobials, antiinflammatory agents) was shown to be beneficial. Horses receiving J5 hyperimmune plasma had a significantly improved clinical appearance 48 hours after plasma administration and a shorter recovery time than did control horses.

**Lipid A analogues** likely bind to (but do not activate) LPS receptors and binding proteins (LBP). Macrophage and neutrophil activation is inhibited.

Lipid X, a monosaccharide precursor of lipid A, was shown to confer a dose-dependent survival benefit in one model of sepsis. Monophosphoryl lipid A is a nontoxic lipid A derivative with weak antagonist properties. Pretreatment with monophosphoryl lipid A reduces cytokine expression. Recently investigated analogues have been shown to have more potent antagonistic effects in animal models of endotoxemia and bacteremia. The efficacy of E5531, a synthetic analogue based on *Rhodobacter* LPS, has not, however, been established in different animal species.

**Bactericidal permeability increasing protein** (BPI), which is of neutrophil granule origin, is a cationic, endotoxin-binding protein capable of damaging the cytoplasmic membrane of both gram-negative and gram-positive bacteria. BPI is released in areas of inflammation. Actions of BPI may be thought of as opposite to LBP. It has the ability to bind to the lipid A region of LPS, preventing neutrophil activation. Recombinant BPI has been shown to inhibit LPS and bacteria-induced inflammatory mediator release.
peting with LBP may be part of the normal modulation of immune responses to endotoxin.

**Polymyxin B** was isolated from *Bacillus polymyxa* in 1947. The polymyxins are reported to be bacteriostatic at low concentrations and bactericidal at high concentrations. The basic structure is that of a polycationic peptide ring containing amino acids with a high proportion of 2,4-diaminobutyric acid residues. A fatty acid side chain is linked to the peptide ring via an amide linkage. Positively charged 2,4-diaminobutyric acid molecules and the fatty acid tail cause the molecule to have an amphipathic nature. As a result, polymyxin B distributes well in both aqueous and non-aqueous environments. Polymyxin B avidly binds the lipid A portion of LPS, attenuating its toxic activities. The beneficial effects of LPS binding by polymyxin B have been shown. Some aminoglycoside antibiotics have also been shown to neutralize endotoxin, but to a lesser degree than polymyxin B.

**Cytokine Antagonism**

Passive immunization with serum-containing antibodies against **TNF-α** significantly protected rats from the lethal effects of splanchnic artery occlusion shock. It also lowered serum TNF-α levels and protected the rats against the impairment in peritoneal macrophage function. There were beneficial effects on the cardiovascular system, and necrosis of the ileum induced by this model of shock was reduced. In some studies, the patients receiving the lowest dose of anti-TNF-α therapy fared the best. Therefore, it should be considered that TNF-α may have beneficial effects in the host's defense against disease, especially against intracellular pathogens.

Naturally occurring inter- **leukin(IL)-1** receptor antagonist is produced during inflammation as part of the innate regulation of the response. Recombinant IL-1 receptor antagonist that blocks the activity of IL-1 and is without detectable agonist activity has been produced.

**Antiinflammatory Therapies**

The inhibition of cyclooxygenase (COX) activity by **NSAIDs** has shown a benefit in many animal models of sepsis. Hypotension can be reversed; temperature, heart rate, and gas exchange can be improved. Both constitutive COX-1 and inducible COX-2 can be blocked. Constitutive functions include the maintenance of mucosal and renal papillary blood flow, and blockade of these leads to detrimental effects on the host.

**Glucocorticoids** are widely used for their immunosuppressive and antiinflammatory effects. They have an inhibitory effect on cytokine production and action and can inhibit the induction of the inducible form of nitric oxide synthase (NOS) in vascular endothelial cells without affecting the constitutive form. Glucocorticoids have little or no effect on constitutive prostaglandin production as they only inhibit COX-2 gene expression. Experimentally, they have been shown to have positive effects when administered before the onset of septic challenge in animal models. No demonstrable benefit in established sepsis has been shown in controlled clinical trials. High doses of steroids have been demonstrated to increase mortality compared with NSAIDs.

The **lazaroids** (21-aminosteroids) are synthetic steroid analogues that have antioxidant and free radical scavenging properties. They have been shown to decrease eicosanoid and TNF-α production and reduce the accumulation of neutrophils. Lazaroids increased survival in animal trials, and their use in humans appears to be safe and without side effects. Availability and cost are the major disadvantages.

**Inflammatory Mediator Antagonists**

**Proinflammatory Strategies**

During sepsis, the immune system can be considered as one more failing organ system. Therefore, treatments aimed at decreasing the immune response may in fact be detrimental to the outcome. Amplification of defenses has been accomplished experimentally by the use of granulocyte colony-stimulating factor (G-CSF) (Figure 3). Colony-stimulating factors are responsible for the proliferation and differentiation of hematopoietic progenitor cells. Of the identified colony-stimulating factors, G-CSF is important in maintaining neutrophil count and responses. G-CSF is found at higher levels in

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**Proinflammatory Strategies**

- Phagocytosis
- Respiratory burst
- Cellular cytotoxicosis
- Microbicidal activity
- Chemotaxis

**Figure 3**—Restoration or amplification of the host defense system by amplification of the proinflammatory response may be an appropriate strategy for the immunocompromised host (i.e., the septic patient).
the organs than in circulation and is increased on exposure to bacterial products and secondary inflammatory mediators. Endothelial cells, fibroblasts, bone marrow stromal cells, and macrophages are stimulated by TNF-α and IL-1 to increase G-CSF secretion. Circulating levels of G-CSF rise rapidly in response to endotoxin infusion and infectious disease. Low tissue concentrations attract neutrophils; high tissue concentrations inhibit motility, serving to attract and then immobilize neutrophils at the site of inflammation. Phagocytic and microbicidal activity is improved. G-CSF has been shown to be more effective than HA-1A in sepsis models.

**Antiinflammatory Cytokines**

Interleukin-10–deficient mice or mice treated with a monoclonal antibody against IL-10 showed higher plasma levels of proinflammatory cytokines and increased mortality. In contrast, administration of recombinant IL-10 to LPS-challenged mice showed protective effects against proinflammatory cytokine production and lethality.

**PHARMACOLOGIC THERAPY**

Conventional treatment for endotoxia and sepsis focuses on identifying the site of infection and controlling it. Supportive therapies include volume replacement, acid–base and electrolyte correction, and the use of pressor agents to maintain tissue perfusion. The current mainstay of therapy for sepsis is the use of antibiotics. Appropriate therapy has been shown to significantly reduce mortality. A concern with the use of bactericidal drugs is the promotion of the release of endotoxin. Supportive steps must be taken to minimize its effects. Cell-wall active antibiotics (e.g., β-lactams, quinolones, aminoglycosides, imipenem) release large quantities of endotoxin by their modes of action. Bacteriostatic antibiotics are inferior to bactericidal antibiotics in their ability to reduce the number of viable bacteria and should not be chosen over them in an effort to minimize endotoxin release. Alternate strategies include delaying treatment until the patient is hemodynamically stable or initiating treatment with lower doses of antibiotics. Use of a chelating agent or anti-LPS antibodies before antibacterial therapy has also been proposed.

The eicosanoids are central to the inflammatory cascade. Flunixin meglumine is the most widely used NSAID in equine practice. In experimental sepsis models, pretreatment with flunixin meglumine (1 mg/kg) prevented most endotoxin-induced changes and correlated with a significant decrease in plasma thromboxane B₂ and prostacyclin concentrations compared with control horses. Elevations in blood lactate were significantly suppressed in horses pretreated with 0.25 mg/kg flunixin meglumine. Low doses of flunixin meglumine inhibit eicosanoid production without masking all physical manifestations of endotoxia. Physiologic activity of flunixin meglumine in foals appears similar to that in mature horses, although ulcerogenic potential is increased. Pretreatment with phenylbutazone (2 mg/kg) attenuated the effects of endotoxin. Other NSAIDs have been used in equine practice (e.g., ketoprofen, ibuprofen, dipyrone, aspirin, indomethacin), and many have also been investigated for beneficial effects in endotoxia. None of the available NSAIDs has shown positive effects when used in animals with preexisting sepsis.

In both human and animal models of sepsis, pentoxifylline, a methylxanthine derivative, and its congeners have been shown to exert numerous beneficial effects, including improved hemodynamics, inhibition of platelet aggregation, increased erythrocyte and leukocyte deformability, enhanced prostacyclin release, decreased thromboxane release, inhibition of endothelial cell activation, suppression of mediator production, decreased lactic acid production in skeletal muscle, and altered leukocyte adhesion. These effects have been attributed to the inhibition of cellular phosphodiesterase with a resultant increase in cAMP concentration. These actions combine to enhance bacterial clearance, improve aerobic metabolism, and counter inflammation. Pentoxifylline is dosed orally at 8.5 mg/kg q8–12h. Intermittent doses of pentoxifylline may allow a beneficial mixture of proinflammatory and antiinflammatory effects.

The polymyxins have been shown to have useful antiendotoxin activity. They are soluble in water, blood, and lipid cell membranes. Polymyxin B does not distribute well into pleural or synovial cavities, nor does it reach useful cerebrospinal fluid concentrations. When administered to humans and animals, polymyxin B binds by its free amino acid groups to negatively charged phospholipids in tissues. This binding has been shown to be greatest in the brain and kidney, with lesser amounts in the liver, lung, and muscle. The most significant toxicity involves the nervous system and kidneys.

Clinically, polymyxin B sulfate is given at the rate of 1 mg/kg (6000 IU/kg) IV q8–12h. Frequency is dependent on clinical judgment. Use of polymyxin B with an NSAID has been experimentally suggested as a viable treatment for endotoxia; the NSAID ameliorates some of the proinflammatory effects of polymyxin B. Concurrent use of a cell-wall active antibiotic has been shown to improve clinical outcome.

The ability of polymyxin B to bind and inhibit the activity of lipid A has spurred the development of con-
jugates and derivatives of reduced toxicity. Polymyxin B nonapeptide is a polymyxin B derivative that is less toxic but also less effective than polymyxin B. The conjugation of polymyxin B prolongs half-life and reduces delivery to the renal tubules, a site of toxicity. Extracorporeal techniques are available to avoid toxicity.

In veterinary medicine, nonspecific enhancement of the defense mechanisms of the body relies on the use of exogenous immunostimulants of bacterial, viral, and plant origin. Macrophage activation and the subsequent release of cytokines (e.g., TNF-α, IL-1, interferon-γ) boost cellular and humoral immune function. Preparations are chiefly used for the treatment of chronic bacterial and viral infections. Mycobacterial cell-wall extracts, levamisole, and interferon-α have all been used.

**FUTURE AREAS OF RESEARCH**

In addition to anti-LPS or anticytokine agents, several strategies interfering with the sepsis cascade have been attempted clinically. These trials include studies with complement factor inhibitors, prostaglandins (e.g., liposomal prostaglandin), antiprostaglandin therapy (e.g., ibuprofen), bradykinin antagonists, platelet-activating factor antagonists, and coagulation inhibitors (e.g., protein C, antithrombin III). Clinical trials are ongoing but have not clearly demonstrated benefits.

Nonselective inhibition of NOS inhibits the activity of endothelial cell constitutive NOS, potentially increasing organ hypoperfusion and ischemia and decreasing cardiac output. The formation of nitric oxide by inducible NOS leads to hypotension, loss of vascular response to vasoconstrictive substances, and depressed cardiac performance. Therefore, agents that selectively inhibit inducible NOS activity may have beneficial therapeutic effects.

CD-14 has been shown to be essential in the recognition of LPS and triggering of cellular activation processes. Blocking of LPS-CD14 binding by anti-CD14 monoclonal antibodies has been shown to protect against organ injury and death, even when administered after LPS exposure. Therapeutic benefit may, therefore, be possible in the face of cumulative LPS exposure.

**CONCLUSION**

Traditional therapies to counter endotoxemia and sepsis have been supportive and reparative in nature. These therapies have centered on the restoration of circulatory volume and tissue perfusion, with the concurrent use of antibacterials and antiinflammatory agents. Experimentally, the preemptive use of many compounds has been shown to have a beneficial effect on the morbidity and mortality of challenged animals. Clinically, few compounds are thought to be of value to septic patients, and these are empirically used. Newer compounds offer greater selectivity of action but are clinically unproven. As yet, there is no universal solution to the problems of endotoxemia and sepsis, and use of any agent depends on the clinician, assessment of each individual case, and constraints to its management.

**REFERENCES**


1. Which of the following statements regarding E5 antibody is true?
   a. It reduced endotoxin levels experimentally.
   b. It improved mortality rates in humans with preexisting sepsis.
   c. It did not improve survival with preemptive usage.
   d. It is of human origin.

2. The use of HA-1A antibody
   a. consistently improved survival in trials.
   b. neutralized endotoxin.
   c. prevented LPS-induced B-cell proliferation.
   d. did not inhibit LPS-induced cytokine secretion.

3. BPI
   a. is incapable of binding lipid A.
   b. is of neutrophil granule origin.
   c. does not prevent neutrophil activation.
   d. is synergistic with LBP.

4. Glucocorticoids
   a. have an inhibitory effect on cytokine production.
   b. suppress constitutive NOS.
   c. inhibit COX-1 expression.
   d. have proven benefits in established sepsis.

5. Lazaroids
   a. increase eicosanoid production.
   b. increase neutrophil accumulation.
   c. have antioxidant and free radical scavenging properties.
   d. stimulate TNF-α release.

6. G-CSF
   a. depresses phagocytic activity.
   b. is increased on exposure to secondary inflammatory mediators.
   c. depresses neutrophil counts.
   d. is chemotactic at high tissue concentrations.

7. Conventional therapy for endotoxemia includes
   a. volume replacement.
   b. acid–base correction.
   c. pressor agents.
   d. all of the above

8. In sepsis experiments involving preemptive flunixin meglumine,
   a. blood lactate was significantly increased.
   b. thromboxane A₂ levels were decreased.

9. Pentoxifylline has been shown in animal and human models of sepsis to
   a. increase skeletal muscle lactate production.
   b. promote platelet aggregation.
   c. decrease cAMP concentration.
   d. enhance prostacyclin release.

10. Polymyxin B
    a. is poorly soluble in lipid membranes.
    b. distributes well into the synovial cavities.
    c. binds the best to brain and kidney.
    d. clinical efficacy is not improved by concurrent use of a cell-wall active antibiotic.