REVIEW ARTICLE

Drugs 1998 Apr; 55 (4): 497-508
0012-6667/98/0004-0497/$12.00/0

Antiendotoxin Strategies for the Prevention and Treatment of Septic Shock

New Approaches and Future Directions

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Contents

Summary 497
1. Specific Antiendotoxin Strategies 499
   1.1 Inhibitors of Lipid A Biosynthesis 499
   1.2 Antiendotoxin Antibodies and Vaccine Strategies 501
   1.3 Bactericidal/Permeability-Increasing Protein 502
   1.4 Reconstituted High Density Lipoprotein 502
   1.5 Lipopolysaccharide (LPS) Antagonists 503
   1.6 Anti-CD14 Antibodies 505
   1.7 LPS Signal Transduction Inhibitors 505
2. Conclusions 505

Summary

Therapy for Gram-negative sepsis remains unsatisfactory despite a concerted effort to develop new treatments for this common, life-threatening syndrome. Current research continues on several fronts to improve the treatment options available to clinicians in the management of these critically ill patients. Recently, a greater understanding of the complex molecular basis of endotoxin-mediated pathophysiological effects in humans has generated a number of novel therapeutic agents for sepsis. Several of these treatment strategies have already entered clinical trials and it is hoped that some of these therapies will become widely available in the near future.

In this review, the current status of the most promising new antiendotoxin agents is summarised, and the major obstacles to the successful clinical development of these therapies are described. New antiendotoxin therapies include those which interrupt the synthesis of endotoxin, bind and neutralise its activity, prevent endotoxin interactions with host effector cells and interfere with endotoxin-mediated signal transduction pathways. Potential therapeutic strategies involving these agents consist of endotoxin analogues, antibodies, subunit vaccines, binding columns, recombinant human proteins and small molecule inhibitors of endotoxin synthesis and intracellular signalling. The pitfalls of previous antiendotoxin clinical investigations and the perils of future clinical trial designs are discussed in the context of unmet needs and realistic expectations for success.

While considerable progress has been made, effective and new treatments for Gram-negative bacterial sepsis continues to elude us at the present time. This has

been to the detriment of patients, investigators and pharmaceutical companies alike. It will require focused efforts by basic scientists, continued support by industry and enlightened study designs by clinical investigators to successfully develop antiendotoxin therapies for use in septic patients in the future.

Bacterial endotoxin (also referred to as lipopolysaccharide or LPS) remains an important target for new drug development in the treatment of serious infections from Gram-negative organisms. Despite recent, well documented failures of anti-endotoxin monoclonal antibody therapies for septic shock,[1-3] a critical need remains for improved treatment strategies for systemic infections from Gram-negative bacterial pathogens. The overall mortality rate from septic shock caused by Gram-negative organisms in recent clinical studies remains at approximately 45%.[1-3]

A major contributor to lethality from Gram-negative sepsis is endotoxin itself. This complex, amphiphilic macromolecule is an essential structural component of the outer membrane of Gram-negative bacteria. It is released from the cell wall of bacteria as they grow and as they are lysed by complement, antibodies, antibacterials or phagocytic cells. Endotoxin is a highly toxic molecule that, if introduced accidentally or intentionally into the systemic circulation, will result in a pathophysiological state which mimics bacteraemic, Gram-negative septic shock.[4,5]

Over the course of vertebrate evolution, *Homo sapiens* has become among the most susceptible animal species to the lethal effects of bacterial endotoxin. The only other mammals that approach a similar level of endotoxin sensitivity are horses and chimpanzees. This profound sensitivity to the pathological consequences of bacterial endotoxin presumably had some survival advantage for our early hominid ancestors; however, it has become a liability in modern medicine as we have excellent antimicrobial agents against most Gram-negative bacteria but no effective therapy against endotoxin.[2,3,6] In fact, bactericidal antimicrobial agents may paradoxically exacerbate the harmful effects of endotoxin, as the antibacterial may accelerate the release of endotoxin from the outer membrane of Gram-negative bacteria during bacteriolysis. Therefore, treatment strategies which limit the release or the immunological activity of endotoxin may prove to be useful in the management of patients with systemic bacterial infections.[4]

Endotoxin is essentially an alarm molecule that alerts the vertebrate host to the presence of an invasive Gram-negative bacterium within the body. This warns the host of the necessity to activate appropriate innate and adaptive immune defences in an effort to clear the microbial pathogen. This physiological defence mechanism has evolved as a survival strategy to localise, contain and eradicate invading bacterial pathogens. Unfortunately, the same immunological responses to endotoxin which serve to protect the host in localised bacterial infections may precipitate a generalised and potentially fatal systemic inflammatory process in the presence of bloodstream infection from Gram-negative organisms.[2-8]

The endotoxin molecule itself is not intrinsically toxic; it is the exaggerated host response to the systemic release of endotoxin that accounts for septic shock from Gram-negative bacterial organisms. Endotoxin mediates its injurious effects through systemic activation of host-derived inflammatory mediators which include: (a) the pro-inflammatory cytokine networks; (b) neutrophil, monocyte and endothelial cell activation; (c) the complement system; (d) the extrinsic coagulation cascade and the fibrinolytic system; (e) platelet activating factor; (f) the kinins; (g) the prostaglandins and leukotrienes; (h) reactive oxygen intermediates; (i) nitric oxide; and (j) probably others as yet undetermined.[4-8] These inflammatory mediators combine to precipitate endotoxic shock in the presence of systemic Gram-negative bacterial infection.

The precise role that endotoxin itself plays in the generation of multiorgan failure and septic shock

remains the subject of some controversy.[2-5] The evolution of organ dysfunction in septic shock is a complex, highly variable and multifactorial process involving many mediators. It is abundantly clear that endotoxin itself is not absolutely required for the full expression of septic shock in humans. Certain exotoxins such as staphylococcal enterotoxin B or toxic shock syndrome toxin-1 from Gram-positive bacteria (which are devoid of endotoxin) function as superantigens that may precipitate a syndrome which is indistinguishable from Gram-negative septic shock.[6] However, purified endotoxin itself is sufficient to induce systemic inflammation and shock in humans.[5] Thus, therapeutic interventions which interfere with the recognition of endotoxin in the circulation, or contribute to its removal, continue to be sought for the prevention and treatment of septic shock. Ultimately, antiendotoxin treatments will need to be combined with agents effective in the pathogenesis of Gram-positive bacterial sepsis, and possibly fungal sepsis as well.

A great deal of recent information about the synthesis and metabolic handling of endotoxin has rekindled interest in endotoxin as a primary target for new drug development in the treatment of septic shock.[9,10] Despite recent disappointments with antiendotoxin antibodies, it is possible that agents with enhanced endotoxin-neutralising capacity might be efficacious where less active agents have failed. Moreover, the unsuccessful clinical trial experiences with antiendotoxin therapies in the past should assist investigators in the design of clinical studies which avoid some of the pitfalls of earlier attempts.[3,6]

Numerous antiendotoxin strategies are in various stages of preclinical and clinical development at the present time. These new agents are the focus of this brief review. The basic mechanisms by which endotoxin mediates its pathological effects are outlined in figure 1. Therapeutic strategies which target each of these mechanisms are also highlighted in this figure.

1. Specific Antiendotoxin Strategies

1.1 Inhibitors of Lipid A Biosynthesis

Endotoxin is an essential structural element of the outer membrane of Gram-negative bacteria. Mutations in the biosynthesis of LPS render these bacteria susceptible to clearance and lysis by complement components.[10] A bacteriophage has recently been isolated which infects and disrupts enteric Gram-negative bacteria through the inhibition of LPS biosynthesis. The phage produces a short nucleotide sequence which functions as an antisense RNA which blocks the production of bacterial enzymes responsible for LPS synthesis.[11] This suggests that agents that interfere with endotoxin synthesis could be developed as novel antimicrobials against Gram-negative bacteria. Such agents would function much in the same manner as penicillins, which disrupt the synthesis of another essential cell wall constituent (i.e. peptidoglycan).

Previous studies have identified the different enzymes involved in the biosynthesis of bacterial lipid A. A strain of *Escherichia coli* mutants defective in UDP-*N*-acetylglucosamine acyltransferase, the first enzyme in the lipid A pathway, demonstrated a decline in growth, viability and rate of synthesis of lipid A by 10-fold when exposed to a higher temperature (42°C).[12] This same mutant was tested against rifampicin (rifampin), erythromycin, clindamycin and fusidic acid and was found to be highly susceptible.[13]

Another study focused on UDP-3-*O*-acyl-*N* acetylglucosamine deacetylase, the second enzyme and the first committed step in the lipid A biosynthetic pathway encoded by the gene *envA*.[14] *E. coli* strains with mutations within this gene have less deacetylase activity (an 18-fold reduction) with the level of the enzyme present in direct relation to the degree of *envA* gene expression.[15]

A more recent study identified agents that could inhibit the deacetylase.[16] L-573655, a chiral hydroxamic acid attached to a 2 phenyloxazoline ring system, competitively inhibited the biosynthesis of LPS by 80 to 90%. Several analogues of

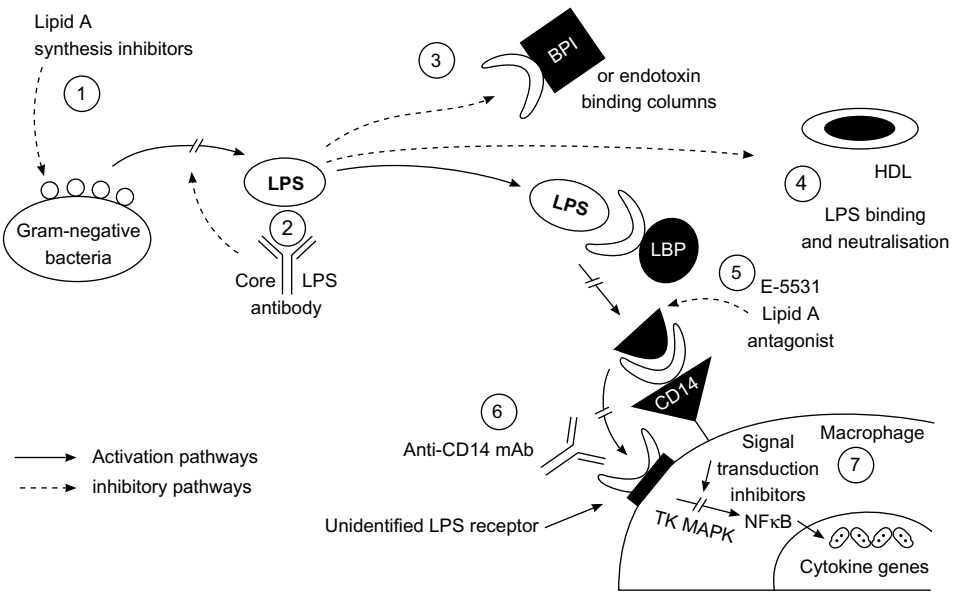

Fig. 1. Schematic representation of various pathways of endotoxin activity and the 7 potential points of intervention discussed in this review. (1) Inhibitors of lipid A synthesis; (2) antibodies to the core glycolipid region of lipopolysaccharide (LPS); (3) bactericidal/permeability-increasing (BPI)-protein or endotoxin binding columns to bind and neutralise endotoxin; (4) high density lipoprotein (HDL) to adsorb and clear endotoxin; (5) endotoxin antagonists such as LPS analogues – E-5531; (6) monoclonal antibodies to the LPS attaching membrane protein on monocytes and neutrophils – CD14; (7) intracellular signalling pathway inhibitors. *Abbreviations:* LBP = lipopolysaccharide binding protein; mAb = monoclonal antibodies; MAPK = mitogen-activated protein kinase; NFκB = nuclear factor κ for B cells (important signal transduction protein for cytokine genes in monocytes and neutrophils); TK = tyrosine kinase.

L-573655 were synthesised and tested against *E. coli*. Treatment with these agents was rapidly bactericidal within 4 hours.[16] The analogue, L-161240, demonstrated greater activity against bacterial cells than L-573655, had comparable efficacy to ampicillin and had considerable activity against other Gram-negative bacteria such as *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Proteus mirabilis*. No activity was noted against *Pseudomonas* or *Serratia* spp. L-161240 protected mice from an otherwise lethal intraperitoneal infection with *E. coli* when administered immediately and 6 hours after the bacterial challenge.[16]

The third enzyme in the pathway, UDP-3-*0*-(*R*-3-hydroxymyristoyl)-glucosamine *N*-acyltransferase, is encoded by the *firA* gene.[17] *E. coli* strains with mutation in this gene had lower enzyme levels, resulting in a 5-fold decrease in the rate of lipid A biosynthesis at 43°C compared with more permissive temperatures (30°C).

Competitive inhibitors of 3-deoxy-D-*manno*-octulosanate cytidyltransferase (CMP-KDO synthetase) have been synthesised and studied. CMP-KDO synthetase activates 3-deoxy-D-*manno*-octulosanate (KDO) for incorporation into LPS.[18,19] Inhibition of the enzyme resulted in the accumulation of lipid A precursors and eventually in bacterial killing. Pretreatment with the KDO inhibitor resulted in killing of >95% of *E. coli* in the presence of serum, indicating that the inhibitor increases bacterial susceptibility to complement-mediated lysis.[18] Bacterial sensitivity to antibacterials increases 10-fold after pretreatment with the inhibitor; thus, lower doses of antibacterials are needed to derive the same therapeutic benefit against Gram-negative bacteria.

As LPS serves a vital function in Gram-negative bacterial growth and virulence, the preceding studies demonstrate that inhibitors of LPS (or lipid A) biosynthesis can result in decreased viability,[12,16]

and increased susceptibility of these organisms to antibacterials.[18] Enhancing the ability of antibacterials to permeate the outer membrane of Gram-negative bacteria may allow lipid A biosynthesis inhibitors to complement the activity of antimicrobial agents directed against these microbial pathogens.

1.2 Antiendotoxin Antibodies and Vaccine Strategies

Antiendotoxin monoclonal antibodies (mAbs) have been studied extensively in the laboratory and in large clinical trials throughout the 1990s. Antibodies directed towards the highly conserved, core glycolipid structure of endotoxin (lipid A and a short sequence of core oligosaccharides) were expected to provide broad cross-protection against endotoxins from a variety of pathogenic Gram-negative organisms.[20] The clinical trials with both edobacomab[1,8] and nebacumab (HA-1A)[7] anti–lipid A mAbs have ended with essentially negative results. The explanations for the failure of these promising and much anticipated antibodies to significantly improve the outcome of Gram-negative bacterial sepsis have been the subject of many reviews and editorials.[2,3,10]

It is indeed unfortunate that other antiendotoxin compounds with much greater specific activity will be difficult to develop as a result of the early unsuccessful trials with edobacomab and nebacumab. These antibodies had rather low intrinsic binding affinities to the lipid A component of bacterial endotoxin.[21,22] Nebacumab binds to other antigens found on human B cells and red blood cells[23] and proved to be toxic in a canine model of bacterial sepsis.[24] Further clinical development of these antibodies has been discontinued.

Despite these setbacks, recent clinical findings have provided additional evidence that anti–core glycolipid antibodies may, in fact, benefit patients with critical illnesses. Goldie and co-workers[25] have shown that pre-existing immunoglobulin (Ig) M anti–core glycolipid antibody levels are associated with improved outcome in septic patients. Nys et al.[26] reported that patients who undergo major surgery were less likely to succumb to sepsis if they maintained high levels of endogenous anti–core glycolipid antibodies. Rietschel and colleagues[10] have conducted a detailed analysis of the physicochemical properties of LPS and examined relevant epitopes within the core glycolipid structure of endotoxin. Their group has defined core glycolipid epitopes which are recognised by the immune system in the presence or absence of serotype-specific O side-chains of polysaccharides on the outer part of the LPS molecule.

Antibodies have been isolated and developed which bind with high affinity to endotoxin from a variety of Gram-negative bacterial pathogens. A chimeric IgG_1 monoclonal antibody known as SDZ 219-800[27] has been developed which may prove to be an effective therapeutic agent in human endotoxaemia. Whether this antibody or similar anti–core glycolipid antibodies will be further investigated in large phase III clinical trials remains to be seen.

Another potential antiendotoxin antibody approach is through the development of an active immunisation strategy. Work on vaccines against the conserved core glycolipid of bacterial endotoxin has recently yielded a preparation which appears promising. A detoxified LPS vaccine (using deacylated LPS from the *E. coli* J5 galE mutant) combined with an outer membrane protein from group B meningococcus has been shown to be highly immunogenic and well tolerated in experimental animal systems.[28] Polyclonal antibodies produced in response to the vaccine bind to the core regions of LPS molecules of a number of Gram-negative bacilli and protect animals from lethal endotoxin challenge and invasive Gram-negative infections.[29]

An active immunisation strategy has several theoretical advantages. Among these are low production costs, the ability of the vaccine to generate high titre endogenous antibodies in recipients, and the opportunity to use the vaccine as a preventative agent. A prophylactic treatment would be desirable in that humans respond precipitously to endotoxin with activation of a complex array of systemic in-

flammatory mediators. It is difficult clinically to detect the early signs of endotoxaemia in time to effectively intervene with a specific antiendotoxin therapy.[3,6] A vaccine would stimulate the production of antibodies before and during the period of systemic endotoxin release and provide protection at the critical initial time period of early Gram-negative sepsis.

Lastly, it should be pointed out that another potential antiendotoxin antibody strategy would be the provision of polyclonal antibody protection against O-specific polysaccharide side-chain antigens. A limited number of common Gram-negative bacterial serotypes cause the majority of human bloodstream infections. Donta and co-workers[30] have prepared a polyclonal hyperimmune human serum treatment against common strains of *Klebsiella* and *Pseudomonas* spp. Clinical trials with this polyvalent antiserum have met with limited success in selected patients but did not significantly benefit the entire study population.

1.3 Bactericidal/Permeability-Increasing Protein

Bactericidal/permeability-increasing protein (BPI) is an endogenous human protein found principally in the primary granules within neutrophils. This 456 amino acid protein has antibacterial actions and also binds with high affinity to the lipid A component of endotoxin.[31-33] It is structurally similar to LPS binding protein (LBP). Both proteins bind with high affinity to the lipid portion of LPS. However, these 2 endotoxin binding proteins differ functionally in that BPI inhibits LPS activation of CD14 bearing effector cells (monocytes, macrophages, and neutrophils) while LBP promotes LPS activity in these immune effector cells. BPI and LBP may act as molecular antagonists in biological fluids which compete with each other for LPS binding.[34,35] The net physiological effects of endotoxin release may depend upon the relative tissue concentrations of these 2 endotoxin binding proteins. LBP predominates in the systemic circulation as it is a hepatic acute phase protein.[36] BPI predominates in abscess cavities as neutrophils degranulate and release their intracellular contents into a site of inflammation.[37]

The 3-dimensional structure of BPI has recently been solved.[38] It forms a V-shaped molecule with planar symmetry between the carboxyl terminus domain and the amino terminus domain. Multiple amino acids form a hydrophobic pocket into which the lipid A portion of the LPS molecule fits. BPI binds to endotoxin with high affinity and effectively removes endotoxin from the circulation. The recombinant holoprotein and the 23 kDa amino terminal portion of BPI have both proven to be remarkably successful in endotoxin challenge experiments in human volunteers[33,34,39] and Gram-negative bacterial challenge experiments in animal models. The protein also has some antibacterial properties as it increases the permeability of Gram-negative bacterial outer membranes which may be lethal to many enteric bacteria.[33] This provides the appealing opportunity to treat with an endogenous human peptide that functions as an antibacterial as well as an endotoxin-neutralising molecule.

The N terminal domain of human recombinant BPI is now in extensive clinical trials in meningococcal sepsis, in haemorrhagic states and in partial hepatectomy. Initial clinical trials in children with meningococcal infections with septic shock were very encouraging in that the mortality rate in treated children was considerably lower than what would be predicted for this severely ill population.[40] One potential problem with this recombinant protein is its short serum half-life (2 to 4 minutes). This necessitates administration of high doses of BPI by a continuous intravenous infusion. Recombinant derivatives of BPI with more favourable pharmacokinetic properties are available if current BPI products prove to be successful in ongoing clinical investigations.

1.4 Reconstituted High Density Lipoprotein

High density lipoprotein (HDL) and other serum proteins such as albumin and low density lipoprotein will bind to endotoxin as it is released in the systemic circulation.[41] High density lipopro-

tein has high affinity binding potential with bacterial endotoxin. The HDL-LPS complex is remarkably stable. Once LPS is adsorbed onto an HDL particle, it is effectively removed from the circulation by hepatic clearance mechanisms. In fact, HDL functions as an endogenous LPS clearance system which limits the possible deleterious effects of endotoxaemia. LPS can be delivered to HDL via the activity of LPS binding protein or soluble forms of CD14 which shuttle LPS to HDL. HDL can be viewed as a reservoir which takes up and eliminates LPS as it enters the circulation.[8,42]

If sufficient quantities of HDL are available, LPS activity in the circulation can be attenuated by the endotoxin binding actions of HDL. Transgenic mice which express increased quantities of human apolipoprotein-A1 (the principal protein component in HDL) are protected from the lethal effects of endotoxin challenge. Conversely, apolipoprotein-A1 knockout mice are highly susceptible to the toxic effects of endotoxin challenge.[43] Unfortunately, patients who are critically ill often have reduced circulating levels of HDL.[44] At a time when the human host is at greatest need of an endogenous antiendotoxin system, HDL levels are significantly diminished.

It has been hypothesised that the administration of HDL to septic patients might replenish this LPS clearance system to the benefit of patients with Gram-negative infections. In a phase I trial with LPS challenge in human volunteers, a reconstituted form of HDL from blood donors was remarkably effective in blocking proinflammatory cytokine synthesis and limiting haematological toxicities following LPS injection.[44,45] A phase II clinical trial with reconstituted HDL in patients with peritonitis is planned in the near future.

A strategy to remove circulating endotoxin from the blood of septic patients by haemofiltration has been initiated by Kodama and colleagues[46] in Japan. Removal of endotoxins from the circulation in Gram-negative infection through the use of an extracorporeal filtration system has been performed using different endotoxin adsorbents with varying efficacy.[47-50] Polymyxin B (PMX) has been shown to neutralise endotoxin[51] but is toxic to the CNS and the kidneys when given systemically. Fixing PMX to insoluble fibre (PMX-F) and utilising it as the main component for direct haemoperfusion (DHP)[47,50] prevented polymyxin from being released into the systemic circulation yet enabled it to bind to endotoxin. Animal studies[47,51,52] and phase II clinical studies[50,53] have already been performed with similar promising results.

Direct haemoperfusion with PMX-F protected experimental animals from an otherwise lethal challenge with *E. coli* LPS.[47,52] The treated animals with experimental septic shock and patients with clinical sepsis had lower levels of endotoxin[47,50,51,53] and tumour necrosis factor (TNF); a lesser degree of hyperthermia; better haemodynamic parameters in terms of cardiac index, systemic vascular resistance, heart rate and blood pressure; and less severe abnormalities in blood pH, glucose levels, plasma lactate levels[47] and tissue oxygenation.[50,53] A decrease in the bacterial counts after treatment was also observed and may be due to a more efficient clearance of the organisms by the liver after the endotoxin has been cleared from the circulation[47] or to favourable effects of haemofiltration on superoxide anion generation and opsonic activity.[53] Patients with Gram-positive infections did not respond as favourably as did patients with Gram-negative sepsis.[50,53]

Extracorporeal filtration systems utilising anion sorbent columns and cellulose adsorbents[48,49] have also been demonstrated to decrease the endotoxin levels. However, DHP with PMX-F was found to be more effective than the same procedure utilising an anion exchange resin.[53] Preliminary experience with this endotoxin removal column has been promising and is under more extensive clinical investigation.[54]

1.5 Lipopolysaccharide (LPS) Antagonists

Antagonism of LPS at the tissue receptor level would theoretically inhibit the subsequent toxic chemical and cellular events of endotoxin ex-

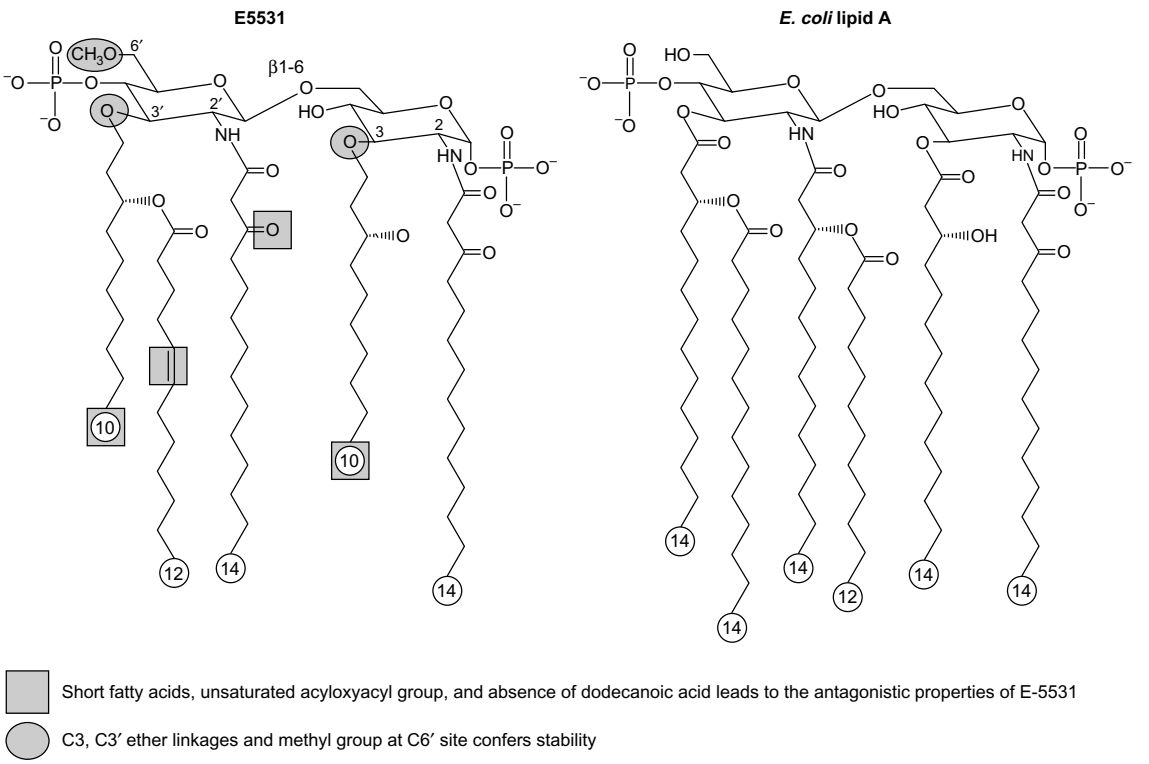

Fig. 2. Molecular structure of E-5531, an endotoxin antagonist; and the corresponding structure of the lipid A portion of *Escherichia coli* endotoxin.

cess.[55,56] E-5531 is an endotoxin antagonist synthesised on the basis of the proposed structure of the lipid A of the bacterium *Rhodobacter capsulatus* (RcLA).[57] The LPS of this nontoxic bacteria has been previously shown to inhibit cytokine production induced by the LPS of other Gram-negative bacteria.[58] E-5531 demonstrated endotoxin antagonism as observed with RcLA; however, it did not exhibit the partial agonistic properties seen with RcLA at high concentrations (up to 100 nmol/L).[59,60] E-5531 was devoid of agonistic activity even in test systems that have been designed to potentiate their sensitivity to agonistic activity.[61] This was achieved by substituting ether linkages for acyl linkages at the C3 and C3′ sites to prevent hydrolytic cleavage that may yield agonistic by-products. Purity and stability was enhanced by blocking the C6 hydroxyl with a methyl group.[62,63] Important structural features of E-5531 as an endotoxin antagonist are shown in figure 2.

The mechanism of action of E-5531 seems to be in its antagonism of LPS activity at its cell surface receptor. This inhibition has been shown to be specific for lipid A induction of cellular activation.[61] Its affinity for binding to cell surfaces is greater than that of *E. coli* lipid A.[59] As the lipid A structure is highly preserved among the different genera of Gram-negative bacteria, E-5531 has been shown to antagonise TNF-α release from human monocytes induced by LPS from *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Salmonella minnesota*.[53] Inhibition of LPS-induced release of interleukin-1 (IL-1), IL-6, IL-8 and IL-10 from human monocytes in whole blood, and nitric oxide from cultured murine macrophages, have also been demonstrated.[53,54]

The greater the amount of LPS in the system, the higher the level of E-5531 needed for optimal inhibition.[53]

In mice injected intravenously with *E. coli* LPS, E-5531 suppressed TNF-α release in a dose-dependent manner and reduced mortality.[61] Another study compared E-5531 with a β-lactam antibacterial (latamoxef, moxalactam) in the treatment of *E. coli* peritonitis in mice.[60] Either E-5531 or the antibacterial alone conferred partial yet transient protection. The use of both agents simultaneously provided prolonged protection and long term survival. With this regimen, only 4 of 30 mice died after 7 days. 26 of 30 mice died when E-5531 was used alone, while treatment with the antibacterial as a single agent resulted in 21 deaths out of 30 animals. The antibacterial decreased the bacterial counts but induced the release of endotoxin from the bacteria during microbial killing. This resulted in antibacterial-induced excess endotoxin release,[62] the deleterious consequences of which were blocked by the addition of the LPS antagonist, E-5531.

As a chemotherapeutic agent for Gram-negative sepsis, E-5531 effectively and specifically antagonises LPS-induced release of mediators in *in vivo* and *in vitro* specimens and in both fixed and circulating cells,[63] thereby decreasing the risk and incidence of death. Treatment of Gram-negative sepsis consisting of antibacterials for microbial killing combined with E-5531 to counteract the endotoxin released from damaged bacterial membranes may prove to be a very effective regimen in clinical care of septic patients. Early clinical investigations with E-5531 in septic patients are ongoing at the present time.

1.6 Anti-CD14 Antibodies

The critical importance of CD14 in monocyte/macrophage signalling has been verified in a number of eloquent studies using recombinant techniques and transgenic animals.[41, 64-70] CD14 knockout mice are resistant to the lethal effects of endotoxin.[70] Since the epitopes which serve as LPS binding sites on membrane and soluble CD14 have been identified,[71-74] it has been possible to develop monoclonal antibodies that block LPS binding to CD14 and signal activation by immune effector cells.[75,76] This potential treatment strategy for human septic shock is under careful preclinical evaluation at the present time.

1.7 LPS Signal Transduction Inhibitors

The final point of intervention against LPS-mediated pathological events is at the intracellular signal pathway level in target cells. The intracellular signal mechanisms for LPS activity in monocytes and neutrophils are complex and highly integrated with other transcriptional activators.[77] The intricacies of these cytoplasmic and nuclear signal mechanisms are the subject of intense investigation in laboratories throughout the world.[9,10,76,77] Some potential therapeutic agents have already been generated from this work. It is clear that a number of tyrosine kinases and mitogen activated protein (MAP) kinases are involved in LPS intracellular signal transduction. Inhibitors of tyrosine kinase have been shown to be protective in experimental models of Gram-negative sepsis in canine peritonitis.[78] It is hoped that similar, or more LPS-specific, signal transduction pathway inhibitors may ultimately prove to be efficacious in human Gram-negative sepsis.

2. Conclusions

Despite early disappointments with antiendotoxin monoclonal antibodies, treatment strategies directed against bacterial endotoxin remain an active area of biomedical research. This approach remains viable as this microbial mediator is the major contributor to septic shock caused by Gram-negative bacteria in humans. Septic shock continues to present a major challenge to clinical medicine since treatment options are limited and the risk of death from sepsis remains unacceptably high.[3,5]

Antiendotoxin treatments have an advantage over most experimental antimediator therapies under investigation for sepsis. Bacterial endotoxin is an unwanted microbial toxin that can be com-

pletely eliminated from the body without potential harm to the patient.[5] This may not be the case with treatments directed against host-derived inflammatory mediators.[79,80]

Key elements of inflammation, such as TNF-α and IL-1-beta, are physiological components of the host defence mechanisms that evolved to facilitate the elimination of invading microbial pathogens. Inhibitors of these proinflammatory cytokines may place the patient at increased risk for overwhelming infection from the very same invading microorganisms that precipitated the septic process. Overly rigorous inhibition of the host immune response has been shown to be actually deleterious in experimental sepsis models with marked exacerbation of virulent microbial infections and rapid lethality.[81]

Antiendotoxin therapies do not jeopardise these advantageous host defence mechanisms. This should allow for the use of antiendotoxin agents as preventative agents or as treatment interventions in the early phases of sepsis where therapeutic agents are most likely to be effective. This will not be feasible with anti-inflammatory agents for the treatment of septic shock.[79]

In the authors' opinion, endotoxin neutralising agents such as BPI or reconstituted HDL along with the endotoxin antagonists (E-5531) represent the most promising experimental antiendotoxin agents at the present time. Other strategies such as bacterial vaccines may prove to be the most efficacious and cost-effective approach to endotoxin inhibition in the future.

Extensive work on a number of experimental approaches directed against endotoxin promises to lead to improvements in the outlook for septic patients. The management of septic shock has not changed substantially for several decades. New treatment approaches are essential to improve the outcome of the common and potentially lethal syndrome of Gram-negative septic shock.

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