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OVERVIEW

Endotoxin elimination in sepsis: physiology and therapeutic application

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Abstract
Purpose The present review summarizes key papers on the elimination of endotoxin in human.
Results Lipopolysaccharides (LPS) are extremely strong stimulators of inflammatory reactions, act at very low concentrations, and are involved in the pathogenesis of sepsis and septic shock. Elimination of LPS is vital; therefore, therapeutic detoxification of LPS may offer new perspectives. Multiple mechanisms eliminate LPS in human comprising molecules that bind LPS and prevent it from signaling, enzymes that degrade and detoxify LPS, processes that inactivate LPS following uptake into the reticulo-endothelial system, and mechanisms of adaptation that modify target cells responding to LPS. These mechanisms are powerful and detoxification capacity adapts as required. Results of therapeutic interventions aiming at the removal of LPS by medication (immunoglobulins) or extracorporeal means are controversial. At least in part, animal experiments revealed increased survival. Human trials confirmed the positive effects on parameters of secondary importance, but not on morbidity or survival which was attributed to the heterogeneity of patients suffering from consequences of severe infectious diseases and sepsis.
Conclusion The hypothesis of LPS-driven inflammatory processes remains very attractive. However, few therapeutic yet immature options have been developed to date.

Keywords Lipopolysaccharide · Elimination · Detoxification · Degradation · Adsorber

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Introduction

Lipopolysaccharide (LPS) or endotoxin is a major constituent of the outer cell wall of especially Gram-negative bacteria and is extremely toxic. Intravenous doses as low as 1 $ng \cdot kg^{-1} \cdot h^{-1}$ cause inflammatory response in humans [1]. LPS is released from the cell wall of growing bacteria or when antibiotics or the complement system destroy bacteria. Upon entering the blood circulation, it may induce systemic inflammation and sepsis, which has a fatal outcome in many cases. Septic shock is characterized by hypotension, fever, disseminated intravascular coagulation, and multiple organ failure. In both experimental and clinical studies, LPS was shown to play a major role in the development of sepsis. LPS interacts with a variety of plasma proteins and activates various immune competent cells. On activation, these cells produce a variety of pro-inflammatory mediators, e.g., tumor necrosis factor (TNF), interleukin-1 (IL-1), nitric oxide (NO), IL-6, and IL-8. It is commonly accepted that excessive systemic release of endogenous pro-inflammatory mediators is of major importance in the pathogenesis of sepsis [2]. Therefore, endogenous and therapeutic removal of LPS and/or molecules mediating the biologic activity of LPS was investigated in order to reduce LPS-initiated consequences. This manuscript highlights mechanisms of the host which deactivate, detoxify,

and eliminate LPS as well as therapeutic measures used for extracorporeal LPS removal.

Materials and methods

This is a review of the English literature cited in the NIH database PubMed using the keywords endotoxin, lipopolysaccharide, detoxification, adsorption, and elimination.

Results

Sources of endotoxin

Translocation Several reservoirs of LPS and mechanisms contribute to endotoxemia. Translocation is probably most relevant because the surface of the gastrointestinal tract is approximately 400 m^2 and concentration of bacteria increase from approximately 0–10^3 colony forming units (CFU) in the stomach up to 10^{14} CFU in the distal colon. Biologically, this compartment belongs to the outside world and the gut barrier separates it from the sterile host. Translocation is the process by which bacteria and bacterial products especially LPS can cross the gut barrier and can cause endotoxemia.

Septic foci Furthermore, infectious bacterial diseases like secondary peritonitis, pneumonia, and soft tissue infection cause bacteria and bacterial products to enter the systemic circulation and systemic inflammatory response.

Antibiotics Finally, antimicrobial agents kill bacteria, and the released bacterial components can boost endotoxemia and contribute to LPS-initiated effects. A recent review focused on key studies on surgery-associated trauma and subsequent consequences on gut barrier, translocation, LPS recognition, and immune system [3].

Elimination of endotoxin by host mechanisms

Selective pressure for evolution preferred higher organisms capable of coping with bacteria and bacterial products, especially LPS. The main known endotoxin detoxification mechanisms comprise molecules that bind LPS and prevent it from engaging TLR4, enzymes that degrade the lipid A moiety in terms of decreasing its activity, inactivation following uptake into the liver and spleen, and adaptations that modify target cell responses to LPS. The term "detoxification" thus encompasses reversible (e.g., binding, sequestration) and irreversible (chemical) modifications of LPS, as well as indirect mechanisms that neutralize or modulate pro-inflammatory host responses to LPS [4].

Anti-LPS antibodies Antibodies can bind and neutralize endotoxin in human. Many immunoglobulin G (IgG; in humans IgG_2) and immunoglobulin M (IgM) antibodies can bind LPS and form complexes, can activate the classical complement pathway, can be bound to receptors on erythrocytes and macrophages, and may clear into the liver, spleen, or reticulo-endothelial tissues [5, 6]. Binding of LPS using either neutralizing antibodies against endotoxin- or ligand-binding lipoproteins such as high-density lipoprotein (HDL) and bactericidal permeability increasing protein (BPI) have been tested [7, 8]. Unfortunately, many of these therapies either aiming at the direct neutralization or at the inhibition of synthesis of inflammatory mediators initiated by LPS showed little or no efficacy in clinical trials.

LPS binding proteins Proteins can also inactivate LPS. Mannose-binding lectin can prevent LPS from binding to TLR4/MD-2 receptors by activating C3 and increasing clearance similar to IgM. Bactericidal permeability increasing protein (BPI) is stored in neutrophil granules and released when cells undergo phagocytosis or exocytosis. BPI kills Gram-negative bacteria by disrupting the integrity of the bacterial cell wall. BPI prevents LPS binding protein (LBP) from transferring LPS to CD14, favoring the uptake of LPS aggregates by host monocytes and neutrophils in a way that avoids cell activation. Other granule proteins like azurocidin bind LPS and augment LPS-induced TNF, IL-8, and prostaglandin E2 production by monocytes [4]. Lipoproteins quickly bind LPS in plasma, and tissue expressing lipoprotein receptors clear these. This binding inactivates circulating LPS, suggesting lipid A moiety is no longer exposed. LBP also promotes transfer of LPS to lipoproteins [9].

Recently, Domingues et al. [10] published new insights on the molecular action of rBPI(21). The peptide is able to interact with LPS of the outer membrane of Gram-negative bacteria and could translocate to the intermembrane space, enabling the interaction with the inner membrane where it exerts higher antimicrobial activity. LPS molecules aggregate in the bloodstream and form clusters. rBPI(21) interacts electrostatically with these LPS clusters and promotes further aggregation and neutralizing LPS toxicity [10]. Upon interaction with Gram-negative bacteria, rBPI (21) is able to bind to the outer leaflet of the outer membrane, enriched in LPS, by electrostatic forces. Afterwards, rBPI(21) inserts into the LPS-rich membrane and moves to the space between the two bacterial membranes. As both the inner leaflet of the outer membrane and all the inner membrane are rich in PG,

rBPI(21) is able to induce the binding between the two bacterial membranes and their fusion (or hemifusion). Domingues et al. [10] proposed that this fusion is the real cause of the changes in permeability and leakage of bacteria content reported for rBPI(21) and BPI, instead of the simple interaction with LPS, fully explaining at the molecular level both the mechanism of microbicide action of rBPI(21) and the naming of BPI as "bactericidal/permeability-increasing protein."

Deacylation of LPS TLR4/MD2 recognizes lipid A, the most highly conserved structural domain of LPS. Full recognition requires phosphates at positions 1 and 4′ and two acyloxyacyl linkages at the backbone. Extracellular and intracellular (myeloid cells) acyloxyacyl hydrolase degrades LPS into inactive or partial agonist molecules [11–13].

Dephosphorylation of LPS The main toxic moiety of LPS resides within the lipid A part in which the two phosphate groups are essential for many biological activities. In contrast to lipid A, monophosphoryl lipid A is not toxic. In fact, administration of placental alkaline phosphatase (plAP) protected mice from the lethal effects of LPS, likely through an LPS-dephosphorylating activity (survival rate after 6 days of the non-treated group was only 57%, whereas it was 100% for the mice receiving plAP after the LPS injection) [2, 14]. Both extracellular and intracellular dephosphorylation of LPS, for instance by alkaline phosphatase, can detoxify LPS.

Alkaline phosphatase In this context, Bol-Schoenmakers et al. [15] recently reported that oral intestinal alkaline phosphatase (iAP) administration had beneficial effects on severe intestinal epithelial damage, whereas in moderate inflammation, endogenous iAP may be sufficient to counteract disease-aggravating effects of LPS. Consequently, iAP treatment may hold a therapeutic promise in case of severe inflammatory bowel disease.

Liver and reticulo-endothelial system The liver quickly removes LPS from the blood. Initially it is taken up by Kupffer cells and then moved into hepatocytes [16, 17]. Intestine-derived endotoxin is cleared this way as well. Studies by Ruiter et al. [18] confirmed the uptake of intravenously injected LPS by hepatocytes. LPS concentrates in the liver very rapidly and 80% of the injected endotoxin accumulates in this organ within a few hours. This is true for rough (R) forms of LPS, while smooth (S) forms of LPS undergo a gradual accumulation with a maximum concentration (43% of the injected endotoxin) 72 h after injection. LPS is also present in the bile of rats following endotoxin injection and then excreted into the gut. However, LPS-induced hepatobiliary factors (in particular TNF-α) cause toxic effects on the intestine which are abolished by external bile drainage [19]. Interestingly, hepatocytes are involved in the clearance of LPS following their intraportal injection, and Kupffer cells do not contribute to this activity, but relatively little is known about how the liver detoxifies endotoxin [20].

Recent investigations suggested argininosuccinate synthase (AS), a liver cytosolic urea cycle enzyme, to be involved in the process of lipid A and LPS clearance, and the extracellular leakage of AS may also participate in the systemic detoxification [21].

Concentration-dependent activity LBP, BPI, CD14, MD-2, and cortisol can increase or decrease the biological activity of LPS depending on their concentration and the setting. For instance, at low concentrations, LBP can catalyze the transfer of LPS monomers to CD14. At high concentrations, LBP promotes clearance of LPS via a non-activating cellular pathway. Acute phase proteins such as LBP, mannose-binding lectin, and C-reactive protein may bind LPS and prevent it from directly activating cells, other acute phase reactants neutralize proteases, oxidants, and pro-inflammatory mediators that appear in the bloodstream [4].

Chylomicrons Vreugdenhil et al. [22] reported cooperative function between LBP and chylomicrons in the detoxification of toxin from both Gram-positive and Gram-negative microorganisms. Enhanced binding of LPS to LBP–chylomicron complexes in the circulation may prevent cytokine production and most likely enhances their clearance from the circulation. Vreugdenhil et al. [22] further speculated that the production of LBP–chylomicron complexes in the gut may represent a natural defense mechanism against endotoxemia of enteric origin. The chylomicron levels attained by oral feeding seem to be of relevance since postprandial levels of chylomicrons are more potent in LPS neutralization in vitro then physiologic levels of other lipoprotein fractions (HDL, LDL, and VLDL) [22].

Under simulated physiological conditions, the binding of LPS to lipoproteins is highly specific. HDL has the highest binding capacity for LPS, the saturation capacity of lipoproteins for endotoxin far exceeds the LPS concentrations measured in clinical situations, and the kinetics of LPS association with lipoproteins display chemotype-dependent differences [23].

Internalization LPS may be internalized because of bacterial ingestion via alternative mechanisms, in particular via

Fc receptor-mediated endocytosis of antibody opsonized pathogens. The Rho family proteins are key components in the cellular regulation of membrane transport and a model as to how this family of proteins is involved in LPS internalization [24]. Caron and Hall [25] have shown that two distinct mechanisms of phagocytosis exist, which both involve Rho family proteins. The first mechanism is Fc receptor-mediated phagocytosis, which is dependent on Cdc42 and Rac; the other mechanism is complement-mediated phagocytosis, which is mediated by Rho. As Rac proteins mediate NADPH oxidase complex activation and thus the respiratory burst, Rac-mediated phagocytosis and subsequent destruction of LPS-containing bacteria are tightly linked to Rho-like GTPases [24].

Internalization of LPS is important for signal transduction. TLR activation and subsequent signaling seems to originate from the endosomal vesicles of the cell [26]. The exact intracellular routing may have important consequences for the final cellular response to LPS, but this has not been investigated in detail. For instance, LPS can be neutralized through uptake via scavenger receptor-dependent phagocytosis in the liver, which does not coincide with strong inflammatory responses. In macrophages and neutrophils, however, internalization of LPS-displaying bacteria is mainly CD14-dependent and is accompanied by marked pro-inflammatory reactions. Kitchens and Munford [27] found that the initial rate and extent of LPS internalization increased with LPS aggregate size. In the presence of LBP, large LPS aggregates were internalized extremely rapidly (70% of the cell-associated LPS was internalized in 1 min). Smaller LPS aggregates were internalized more slowly than the larger aggregates, and LPS monomers, complexed with soluble CD14 in the absence of LBP, were internalized very slowly after binding to membrane CD14 (5% of the cell-associated LPS was internalized in 1 min). Further processing and degradation of LPS occurs via detoxification by acyloxyacyl hydrolase. This enzyme is able to remove the acyl groups from lipid A and thereby detoxifies LPS and its lipid A component [12].

Cybernetics In summary, several biological systems are involved in the removal and activity control of LPS. Local and systemic inflammatory reactions are involved to cope with LPS-initiated consequences. Reticulo-endothelial organs (liver, spleen, lymph nodes) interact with components of the blood and the central nervous system and constitute elements of immune response (stress adaptation). The crosstalk between the various neuronal, endocrine, and immune systems appears to be primarily mediated via the production of and through interactions with soluble immune and neuroendocrine mediators (testosterone, estrogen, gonadotropin-releasing hormone, ACTH, corticoids, growth hormone and prolactin, leptin, ghrelin, IGF-1, endorphins, and enkephalins) and the sympathetic nervous system. For more details, the reader is referred to a recent review elsewhere [3].

Extracorporeal blood purification

Potential relevance of extracorporeal elimination of endotoxin The degree of endotoxemia following LPS injection into healthy volunteers mimics cardiovascular as well as metabolic effects naturally seen in sepsis and septic shock, and in patients with sepsis, the degree of endotoxemia is directly related to mortality [28]. In the critically ill, LPS can contribute to overwhelming the immune system, multiple organ failure, and death. Elimination of endotoxin from the blood may therefore result in improved lethality and morbidity. However, there is no serum threshold concentration for LPS, rendering it deleterious, albeit the higher the LPS concentration, the more severe the consequences. Therefore, it would be rational to eliminate LPS from blood. Nevertheless, extracorporeal LPS removal has major pitfalls, and thus various strategies have been explored to treat patients with life-threatening forms of sepsis.

Adsorber technique Adsorbent-based blood purification is based on adsorption and includes plasmasorption and hemadsorption or hemoperfusion (HP). Adsorption is defined as the removal of molecules by binding on the surface of a material. HP is the direct passage of blood through adsorbent material and is used to remove toxic compounds from the circulatory system. The binding of molecules on the surface of adsorbent material occurs by different processes including hydrophobic interaction, hydrogen binding, electrostatic interaction, covalent binding, or chemical conversion. Because of the complexity of these often coexistent binding processes, adsorbing materials can target molecules in a nonselective, selective, or specific way [29]. Typical adsorbents used in HP devices consist of activated charcoal and charged (ionic) or uncharged resins (nonionic resins).

During sepsis, circulating bacterial-derived products as well as inflammatory mediators can be reduced and/or eliminated by various extracorporeal adjunctive therapies such as plasma exchange, continuous renal replacement, and adsorbent-based therapies. The incorporation of adsorbents in hemoperfusion columns has allowed their use for the removal of toxic compounds from the circulatory system. Adsorbents developed for use in sepsis can bind toxins in a nonselective (e.g., charcoal), selective (e.g., polymyxin B-immobilized polystyrenederivative fiber),

or specific (e.g., antibody-coated microsphere-based detoxification system) way [29].

Polymyxin technique The bactericidal activity of the antibiotic polymyxin B against Gram-negative bacteria is based on its ability to disorganize the bacterial wall after insertion. The cyclic peptide is surface-active and causes dissociation of endotoxin aggregates. Owing to these interactions, polymyxin B has the potential to recognize endotoxins of different origin. Its use in affinity sorbents yields clearance factors (CF) $>10^5$ from heavily contaminated culture filtrates (1–10 μg/ml) of different Gram-negative bacteria. Side effects of polymyxin B comprise nephro- and neurotoxicity, monocyte stimulation (IL-1 release), and protein losses during passage through polymyxin B columns. This is due to the electrostatic attraction of proteins (net-negatively charged) by the ligand (positively charged). This is also the reason why, in spite of 200- to 10,000-fold reduction of endotoxins from plasmid DNA preparations, DNA recovery is only about 50% [30].

Recognition between polymyxin B and endotoxin is mainly due to the interactions of a hydrophobic patch at one side of the peptide and lipid A [31]. It seems, however, that the binding kinetics of this affinity complex are relatively slow as contact times of 16 h are described in batch adsorption experiments. Slow binding kinetics may be the reason that the CFs described in batch experiments can often not be confirmed in column experiments.

Histamine, histidine Ribonucleic acids also interact with endotoxins, and besides the nucleobases adenine, cytosine, etc., the basic amino acids histidine and histamine—the latter being also a potent neuro- and chemotransmitter—have successfully been tested for endotoxin removal from culture filtrates of various microorganisms and were equally effective as polymyxin B [30]. Histidine and histamine show also decontamination potential for various proteins, including serum albumin, insulin, lysozyme, and myoglobin, with CFs ranging from 5 to 200 depending on the protein concentration and environmental conditions. As with polymyxin B, the best protein recoveries and removal efficiencies cannot be achieved independently. The presence of proteins strongly affects endotoxin removal, leading to a more than tenfold reduction of CFs in the presence of BSA and lack of effectiveness in the presence of a murine IgG [30].

Deoxycholic acid Membrane adsorbers based on the ligand deoxycholic acid (DOC) were as effective as others, yielding CFs of approximately 10^4 in the absence of proteins and endotoxin concentrations of approximately 1 μg/ml when DOC was immobilized on a dextran polymer network, located in the flow-through pores [30]. This ligand demonstrated best clearance factors with contaminated fetal calf serum (CF=22) as well as human serum (CF=3.6) and human plasma (CF=2) at initial conditions with 20 mM phosphate buffer at pH 7 and 300 EU ml^{-1} [30].

Polymeric matrices with cationic functional groups Problems with the aforementioned adsorbents lead to the development of synthetic polymeric matrices with cationic functional groups. Amination of spherical porous polymethyl-L-glutamate beads resulted in a better endotoxin-binding capacity than endotoxin adsorbers based on histidine and chitosan. A disadvantage of these adsorbers, however, is the low chemical stability of ester bonds, which was improved by introducing copolymers. This concept allows also the adjustment of the pore size of the beads. The charge density is manipulated by adjusting the ratio of the two monomers. Removal efficiencies were 96–99%, with remaining endotoxin amounts of <1 EU ml^{-1} at 0.5 mg ml of BSA, myoglobin, γ-globulin, or cytochrome *C* and protein recoveries of >99% [30].

Chitosan, a poly-(1,4)-β-D-glucopyranosamine and quaternized chitosan are also referred to as endotoxin-selective ligands. However, the complex formation of endotoxin and chitosan requires an elevated temperature, and again, complex stability seems to be a problem. Furthermore, chitosan triggers monocytes to release TNF-α [30].

Immunoaffinity ligands Given the competing interactions at the surface of sorbents and the existence of proteins in solution, the development of immunoaffinity ligands was another promising idea. Strong efforts were undertaken to develop therapeutic proteins, based on IgG and IgM antibodies, to guard against the problem of endotoxin intoxication, a common problem in hemodialysis and acute bacterial infections. In spite of promising attempts, all clinical trials failed. A reason for these failures is the great variety of chemical endotoxin structures. On the other hand, antibodies raised against the non-polar lipid A, such as HA-1A (Centoxin), displayed mainly non-specific hydrophobic interactions, recognizing hydrophobic moieties of proteins as well. Neither the specific recognition of only one antigen nor the non-specific interactions with proteins are favorable for endotoxin removal from protein solutions [30].

Animal experiments

Kawatsu et al. [32] infused endotoxin (0.05 μg/kg BW from *Escherichia coli* O111:B4) and toxic shock syndrome toxin-1 (TSST-1; 0.3 μg/kg BW) to rabbits as a model for multiple organ dysfunction syndrome and investigated the

effects of the adsorbent CTR with an extracorporeal direct hemoperfusion apheresis system. CTR is composed of porous cellulose beads with immobilized hexadecyl alkyl chains which are capable of removing 50–90% of TNF, IL-1, IL-6, TSST-1, and peptidoglycans in vitro [33]. CTR reduced the mortality rate from 90% to 10% and plasma levels of mediators, TSST-1, and pro-inflammatory interleukins significantly, which coincided with attenuated histopathologic findings in spleen and lymph nodes. Interestingly, endotoxin concentration was below the detection limit in all animal groups with and without adsorber.

Taniguchi et al. [33] did similar experiments but infused solely LPS of *E. coli* (15 mg/kg BW) to rats. Plasmapheresis with CTR reduced the mortality rate from 92% to 14% and removed cytokines, enterotoxins, and TSST-1.

In the only large animal study available so far, Bracht et al. [34] examined the impact of an extracorporeal endotoxin adsorber on hemodynamics, O_2 exchange, and metabolism during resuscitated porcine endotoxemia. Pigs were randomized for intravenous continuous application of LPS of *E. coli* O111:B4 (0.1 μg h^{-1} kg^{-1}) for 8 h or sham. The adsorber in the extracorporeal hemoperfusion device was immobilized human serum albumin. Adsorber therapy was started at the same time as the induction of endotoxemia or 2 h later. Hemodynamic, metabolic, and acid–base parameters, as well as the kinetics of IL-6, IL-8, IL-10, and TNF-α were characteristic for endotoxic shock. Endotoxin plasma levels were low (arterial, hepatic, and portal vein). None of the parameters were significantly influenced by the adsorber system. It was concluded that despite typical clinical signs of endotoxemia, the adsorber system had no significant effect on hemodynamic, metabolic, and acid–base parameters during endotoxic shock. The reasons for the absence of an effect were elusive; however, failure of the method per se or exceeded capacity of the adsorber could not be excluded [34].

Clinical trails

Kojika et al. [35] investigated 24 patients treated for peritonitis and septic shock with endotoxin adsorption therapy using a column of polymyxin B-immobilized fibers (PMX), and their serum endotoxin levels were measured using the high-sensitivity endotoxin assay based on the kinetic turbidimetric Limulus assay. In addition, the changes in the TNF-α production capacity of whole blood following LPS stimulation and clinical outcome in the study patients were also recorded. The 28-day mortality rate was 12%. PMX-direct hemoperfusion (PMX-DHP) was associated with elevation of the mean arterial pressure and urine output, reduction in the mean dose requirement of vasopressor agents, and recovery from shock in all patients. The PaO_2/FIO_2 ratio also showed significant improvement. Using the high-sensitivity endotoxin assay, endotoxin was detected in the blood of 20 out of the 24 patients (80%) before the PMX-DHP, and a significant reduction in the endotoxin level was noted after the PMX-DHP. The TNF-α production capacity of whole blood, which was lower in the septic shock patients than in healthy subjects, was significantly restored after PMX-DHP. Elimination of endotoxin by PMX-DHP in septic shock patients was confirmed by the high-sensitivity endotoxin assay. PMX-DHP was thus considered to be a useful adjuvant therapeutic technique in the treatment of septic shock [35]. There was no relation, however, between the rate of decrease of ET levels and the improvement in the TNF-α production capacity of whole blood following LPS stimulation in this study. Anandamide was also eliminated by PMX-DHP [35]. Ebihara et al. showed that the increased plasma endothelin-1 (ET-1) levels and monocyte ET-1 mRNA levels in patients with sepsis decreased significantly after PMX-DHP [35, 36]. These observations suggested that the elimination of diverse mediators by PMX-DHP might have been involved in the effectiveness of this treatment modality for septic shock. PMX-DHP resulted in the improvement of the sepsis-induced deterioration of pulmonary-oxygenating capacity. Permeability edema, the ventilation–perfusion ratio, etc. also seemed to improve after PMX-DHP. It was suggested that PMX-DHP also eliminated humoral mediators other than ET-1. PMX-DHP might be a useful adjuvant therapeutic technique in the treatment of sepsis [35].

Blomquist et al. [37] studied the effects of a new endotoxin adsorber device (Alteco LPS adsorber) in 15 patients undergoing cardiac surgery because endotoxemia is thought to occur using extracorporeal circulation, and a positive correlation has been proposed between the magnitude of endotoxemia and risk for postoperative complications. Endotoxin was detected in only two patients and IL-1β in four patients. IL-6 levels decreased in both groups, whereas no change in TNF concentrations was found. Since no complications were noted to the use of the adsorber, it was concluded that the Alteco LPS adsorber could be used safely and was easily handled in the bypass circuit. However, the intended effects of the adsorber, i.e., removal of endotoxin from the blood stream, could not be confirmed in this study.

Ullrich et al. [38] reported on the efficient LPS removal by hemoperfusion over high-affinity polymethacrylate-bound albumin (Fresenius Endotoxin Adsorber EN 500) in an open, uncontrolled pilot study on six patients with suspected Gram-negative sepsis. All six critically ill patients improved substantially.

A subsequent phase II trial on 145 patients using the same endotoxin adsorber as Ullrich et al. and Bracht et al. (EN 500, immobilized human serum albumin) in patients

suffering from sepsis or septic shock due to suspected Gram-negative infection, however, failed to show any benefit [39].

ARDS was reported to be associated with endotoxemia, and recently, Nakamura et al. reported that polymyxin B-immobilized fiber treatment reduced blood endotoxin and blood mobility group box-1 protein levels in patients with ARDS significantly [40, 41]. Further details on the clinical utility of PMX-F treatment was very recently published by Nakamura and Yamagishi [42].

Limited validity of LAL test on non-aqueous fluids

The Limulus amebocyte lysate (LAL) test is accepted for the measurement of LPS concentrations in aqueous solutions. However, interpretation of plasma concentrations could be difficult due to possible disruptive factors. A number of proteins show strong interactions with endotoxins, such as LBP, BPI, amyloid P component, cationic protein 18, or the enzyme employed in the biological endotoxin assay (anti-LPS factor from Limulus amebocyte lysate) used in the LAL assay. These proteins are directly involved in the reaction of many different species upon the administration of endotoxin. Other proteins interact as well without having strong links to a biological mechanism, such as lactoferrin or lysozyme. Lysozyme also interferes with the rabbit test, giving false negative results due to masking of endotoxin, a problem occurring also in the LAL test with many—if not all—net-positively charged proteins. In some cases, interaction is not linked to binding but to deaggregation of supramolecular endotoxin structures, for example with hemoglobin or transferrin; de-aggregation usually leads to a higher toxicity of endotoxin in vivo [30].

Finally, it is unknown whether the endotoxin detected in plasma using the Limulus lysate assay is bioactive in vivo (since inhibitors must be removed prior to performing the test) and/or whether quantitation is accurate (a relative newer assay, based on immunodetection of lipid A, may also detect active or inactive molecules) [43].

Future developments

Criteria for the most appropriate endotoxin assay and subsequent development still have to be elaborated. Effective endotoxin-eliminating procedures are missing as well. However, knowledge and technology grow quickly. An example is the recently published optimization of processing and structure of carbide-derived carbons which increased the adsorption rate of the cytokines TNF-α, IL-6, and IL-1β from blood plasma by up to two orders of magnitude [44].

Another field of research is prophylaxis of sepsis with modified LPS molecules. Radio-detoxified endotoxin (RD-LPS) preparations show decreased toxicity, whereas the beneficial effects were preserved [45]. RD-LPS has been tested on 350 surgical patients suffering from gastrointestinal tumors, patients suffering from acquired immunodeficiency syndrome, and cancer patients treated with cysplatin. RD-LPS treatment prevented sepsis and activated the bone marrow function in these patients [45].

Discussion

The poor performance of the anti-endotoxin antibodies raises a general question. Since the circulating blood and the liver already have numerous mechanisms for detoxifying endotoxin, and if those mechanisms become more active as the body responds to infection, is it likely that infusing additional endotoxin-neutralizing drugs will be beneficial in patients with sepsis [4]? It was repeatedly published that patients with detectable plasma endotoxin are at higher risk of developing severe sepsis and poor outcome. Although there is an association between plasma endotoxin and various other pathologies, it is uncertain that neutralizing or removal of this endotoxin would be beneficial. In fact, some authors argued that the detection of endotoxin in isolation had no prognostic value [46, 47].

The same criticism has to be addressed to extracorporeal endotoxin elimination systems. As with all other therapies intended to prevent unwanted inflammation by interfering with innate immune responses to infection, there is a potential pitfall: It is possible that enhancing endotoxin detoxification mechanisms in local tissues will interfere with the protective role of endotoxin sensing in host defense [4]. It was suggested that uncontrolled inflammation within infected extravascular tissues is the key factor in the pathogenesis of severe sepsis and septic shock; in these studies, circulating bacteria did not precipitate shock or systemic injury. These observations raised the possibility that low-level endotoxemia or bacteremia were usually a marker of covert infection and inflammation in an extravascular tissue site, not an independent stimulus to systemic inflammation [4]. If this was correct, it would be possible that enhancing endotoxin detoxifying mechanisms within infected tissue would yield benefit for patients with Gram-negative infections whether or not endotoxemia is detected. Other authors, however, advocated multifactorial therapeutic approaches as a prerequisite to annihilate the deleterious effects of bacterial products, including LPS [29].

Conclusion

Endotoxemia as the fuel of sepsis remains a very attractive hypothesis. To the best of the authors' knowledge, no tool exists to quantify the effects of mechanisms involved in the

elimination and detoxification of LPS neither of the host nor of the therapeutic measures. It is even more difficult to weigh the beneficial effects of endotoxemia. This knowledge would allow for balancing the pros and cons of therapeutic interventions. The puzzle is still fragmentary and even its size is unknown. However, future detailed insights promise major reward.

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