Update on antiendotoxin therapies

Robert Stephens, Colin Hamilton-Davies

Endotoxin has been implicated in the processes that can lead to organ failure and death after surgery and critical illness. While there are no currently available commercial therapies directed against endotoxin, many have been tried or are in an experimental stage. In this article we outline past, present and future approaches to antiendotoxin therapy.

Endotoxin is a component of the Gram-negative bacterial cell wall and can start the processes that lead to organ failure and death. Elevated levels during critical illness, surgery and trauma have been associated with a worse outcome than patients wiht lower endotoxin levels.

There are a number of natural endogenous substances that are thought to neutralize endotoxin, including antiendotoxin antibodies.

Many trials of antiendotoxin substances have failed to show a reduction in mortality in sepsis, and this may be because of the difficulty in intervening once the inflammatory cascade has started.

Current experimental approaches are aimed at stopping endotoxin release, binding free endotoxin, or interfering with the cellular response to endotoxin.

WHAT IS ENDOTOXIN?

Endotoxin, also called lipopolysaccharide (LPS), is found in the outer cell wall of Gram-negative bacteria. It consists of a toxic lipid-A region, inner and outer core sugars and a distinct O polysaccharide side chain (*Figure 1*).

There is a large store of endotoxin within our gastrointestinal tracts in the form of normal bowel flora. As well as this, endotoxin may arise from sites of Gram-negative infection and has also been found in heparin, cardiopulmonary bypass circuits and sterilized infusion fluids. It may be shed spontaneously by bacteria and can be liberated by antibiotics (Shenep and Mogan, 1984).

WHAT CAN ENDOTOXIN DO?

Endotoxin can initiate inflammatory pathways

Endotoxin can initiate multiple inflammatory pathways (*Figure 2*) leading to diffuse microvascular thrombosis, thought to be the cause of the focal structural changes seen throughout organs in multiple organ dysfunction syndrome.

When given to volunteers in small doses, its cardiovascular effects are similar to those seen in sepsis: an increase in heart rate and cardiac index and a decrease in systemic resistance, resulting in a fall in blood pressure (Suffredini et al, 1989). Recipients experience similar symptoms to those in sepsis: headache, chills, myal-

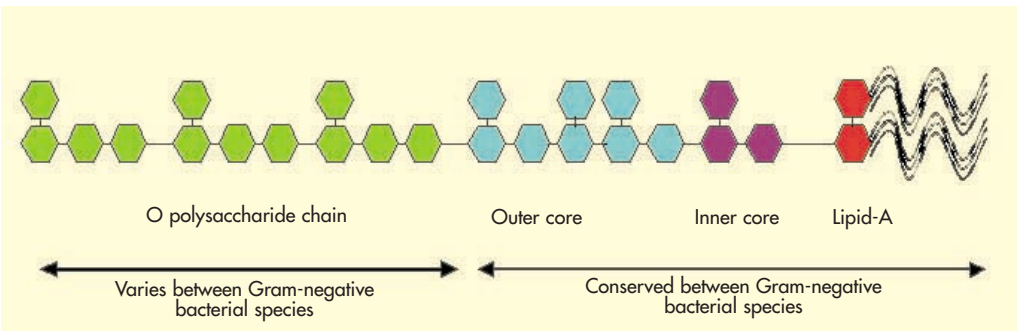

Figure 1. Schematic structure of the endotoxin molecule.

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gia, fatigue, and general malaise in addition to similar cardiovascular, gastrointestinal, immune and haematological changes.

Endotoxin can act on the endothelium to reduce the action of endogenous anticoagulants, to produce free radicals and to increase the production of nitric oxide by inducing nitric oxide synthetase. Monocytes become activated and release cytokines such as tumour necrosis factor α (TNFα), interleukin-1 (IL-1) and interleukin-6 (IL-6), mediators associated with the development of organ failure, while neutrophils cause endothelial damage by releasing superoxide anions and proteolytic enzymes.

Endotoxin and organ failure

Endotoxin has been found in patients undergoing surgery (Foulds et al, 1997), in patients with pancreatitis and during Gram positive, Gram negative and fungal sepsis (Danner et al, 1991); elevated levels being associated with a greater degree of organ failure. According to one theory, this is because during critical illness and surgery underperfused gut mucosa becomes permeable, allowing bacteria or endotoxin contained within the lumen to leak out into the circulation with consequent effects (Pastores et al, 1996). Thus gut-derived endotoxin enters the portal venous system, where in health much of it is taken up by the liver and neutralized by Kuppfer cells.

Endotoxin induces natural antiendotoxin defenses

Being a 'foreign' substance, endotoxin induces natural antibodies to the O polysaccharide chain, core or lipid-A portions. As well as antibodies, several blood-borne substances bind endotoxin such as high density lipoprotein cholesterol (HDL-C) and bactericidal permeability increasing protein (BPI), released from neutrophils. These substances are thought to reduce the effects of endotoxin. On the other hand, lipopolysaccharide binding protein (LBP) removes endotoxin from HDL-C and presents it to CD14, part of the endotoxin receptor on the monocyte surface. This increases the effect of endotoxin (*Figure 3*).

A BRIEF HISTORY OF ANTIENDOTOXIN THERAPIES

The only trial that has shown a mortality reduction with a specific antiendotoxin therapy in sepsis was the groundbreaking study by Ziegler et al (1982). In it, she and her colleagues took 'control' plasma from San Diego firemen before vaccinating them with a mutant J5 *Escherichia coli* endotoxin core–lipid-A preparation and removing more plasma (which acted as treatment plasma). The 'treatment' plasma obtained was given to 191 patients with Gram-negative bacteraemia while 103 patients had the 'control'; the treatment group mortality was 22% compared to 39% in the controls.

Unsuccessful lipid-A monoclonal antibodies

Despite this promising start, the trials of the anti lipid-A monoclonal antibodies HA-1A (Centoxin, Centocor, Malvern, PA, USA) (McCloskey et al, 1994) and E5 (Xoma, Berkley, CA, USA) (Bone et al, 1995) in sepsis failed to show a reduction in mortality on an 'intention to treat' basis. Another monoclonal antibody, T88 (Chiron, Emeryville, CA, USA) (Panacek et al, 1995), also showed no overall benefit. This may be because HA-1A and E5 have little specific ability to bind endotoxin, but it also may reveal the difficulties of attempting to intervene once the inflammatory cascade has been started.

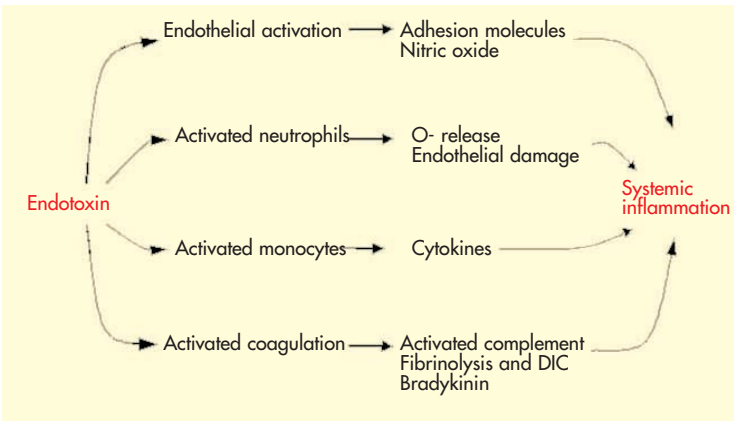

Figure 2. Endotoxin can initiate many inflammatory pathways. DIC = disseminated intravascular coagulation.

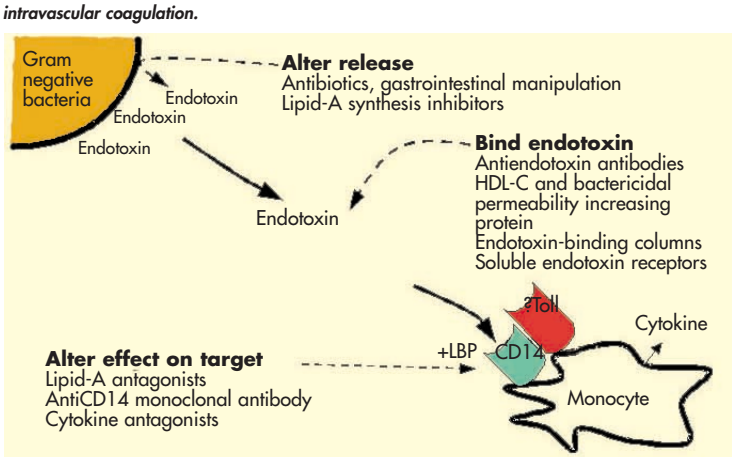

Figure 3. Possible antiendotoxin approaches. HDL-C = high density lipoprotein cholesterol; LBP = lipopolysaccharide binding protein.

Natural antiendotoxin antibodies appear to be protective

Several studies have noted that those patients with higher levels of antibodies to endotoxin 'core' have a better outcome. This finding, in cardiac (Bennett-Guerrero et al, 1997; Hamilton-Davies et al, 1997) and general surgery patients as well as in patients with sepsis (Strutz et al, 1999), has led to a renewed interest in antiendotoxin therapies before exposure.

HOW CAN WE INTERVENE?

Before or after the insult; once the inflammatory cascade has started

Although there is no specific licensed antiendotoxin therapy available, several are in various stages of development. Once endotoxin has been released into the circulation, it can initiate a cascade of events outlined above. For planned insults such as surgery, it is possible, in theory, to give antiendotoxin therapies before endotoxin exposure. In the case of patients with sepsis, who present after endotoxin exposure, this is not possible in the same way.

Stop release of endotoxin

Inhibitors of lipid-A synthesis: As lipid-A is integral to endotoxin's pathological function, altering the enzymes involved in its manufacture will leave the bacterium more prone to lysis and clearance. This can be achieved by either infection with a bacteriophage (a virus that infects a bacteria) that in turn alters the DNA or RNA encoding the enzyme, or by direct enzyme inhibiton of the lipid-A manufacturing enzymes. Both of these approaches are at the preclinical stage.

Antibiotic-induced endotoxin release: As many antibiotics cause bacterial cell wall breakdown, they can also release endotoxin into the circulation. One group that bind to penicillin binding protein 2 (PBP-2), a protein in the bacterial cell wall, is associated with less endotoxin release (Trautmann et al, 1998). Despite the fact that some studies have shown poorer outcomes in those patients with higher endotoxin levels, there is no good prospective trial to show that use of the 'low endotoxin-releasing group' of anti-biotics can result in a better outcome.

Fluid load: If it is the underperfused gut lumen wall that becomes permeable and allows endotoxin to enter the portal circulation, then ensuring adequate gut mucosal perfusion with fluids and vasoactive drugs should result in less endotoxin being released. There is as yet no direct evidence that correcting inadequate gut perfusion reduces endotoxin 'leak'. However, using the sigmoid tonometer as an index of mucosal perfusion, Soong et al (1994) found that lowered sigmoid perfusion was associated with high levels of endotoxin and, in turn, greater levels of postoperative organ dysfunction.

One reason why 'optimization' improves outcome after operations may be that fluid before an operation improves gastrointestinal perfusion (Mythen and Webb, 1995) and therefore less endotoxin exposure may occur as a result.

Selective digestive decontamination: One way to reduce the endotoxin released is to reduce the amount of bacteria present in the gut by selective digestive decontamination. A preoperative oral antibiotic regimen involving polymyxin B, tobramycin, and amphotericin B lowered rectal aerobic Gram-negative bacilli, endotoxin and cytokine levels in patients undergoing cardiopulmonary bypass surgery (Martinez-Pellus et al, 1997), but the effects of this on morbidity or mortality have not been investigated.

Bind free endotoxin

The relationship in some studies between elevated levels of endotoxin and a worse outcome has given rise to the idea that removing 'free' endotoxin is beneficial. This can be done either by raising the level of 'endogenous' endotoxin neutralizing substances (antibodies, HDL-C, BPI and soluble endotoxin receptors) either before or after exposure, or by novel methods such as endotoxin binding columns.

Antibodies: O polysaccharide *vs* core: Antibodies to endotoxin occur naturally and act to neutralize endotoxin, facilitate endotoxin clearance and complement-dependant antibacterial activities, and offer in vivo protection against Gram-negative infection. Antibodies against the O polysaccharide portion will be specific to that species of bacteria: protection is generally limited to that organism. It would be expected that antibodies to the core or lipid-A components, being conserved across a range of Gram-negative bacteria, would show cross reactivity to a range of endotoxins whatever their origin.

Passive *vs* active: There are two ways to increase antibody levels: passively (giving antibodies) or actively (immunizing with antigen). Vaccination requires the patient to be able to mount an antibody response, but if successful, levels are sustained for a greater time than that acquired by passive immunity.

Commercial gammaglobulin: Many commercial gammaglobulins have unknown quantities of antibodies to endotoxin but some have been

found to be useful in both prevention and early treatment of sepsis.

'Natural' hyperimmune plasma: Plasma can be removed from healthy individuals who happen to have high levels of antibodies to endotoxins (Fromsgaard et al, 1988). One study found the resulting hyperimmune gammaglobulin protective in a sheep model of *E. coli* sepsis (Hodgson et al, 1995). Using this approach, a small study in patients with low levels of antibodies to endotoxin core having cardiac surgery found that the preoperative use of hyperimmune plasma shortened the length of hospital stay (Smith et al, 1999). This approach is currently being investigated, although there are concerns about prion transfer in blood products.

Vaccinated volunteer serum: Volunteers have been vaccinated with various 'smooth type' species (endotoxin lacking an O polysaccharide tail) to provide serum rich in antibodies to that bacteria's endotoxin. As mentioned earlier, one mutant, *E. coli* J5, has been effective in both prevention (Baumgartner et al, 1991) and treatment (Ziegler et al, 1982) of sepsis.

Monoclonal antibodies: In theory, a monoclonal antibody against endotoxin would give a plentiful source of infection-free antiendotoxin antibodies, which could be used as either prophylaxis or treatment. However, as described above, the trials of anti-lipid A monoclonal antibodies E5 and HA-1A in sepsis failed prospectively to show a reduction in mortality.

Vaccines against core, lipid-A or O polysaccharide antigens: Vaccines from parts of the O polysaccharide or core (Baumgartner et al, 1991) components have been given to both patients and potential plasma donors. One endotoxin 'core' vaccine that included *E. coli* J5 mutant (Bhattacharjee et al, 1996) was able to protect against endotoxin injection and Gram-negative infection in vaccinated animals. Unfortunately, while human vaccines are immunogenic, they are highly pyrogenic and are thus unpleasant to receive. There are currently no commercially available vaccines that elicit enough antibodies able to crossreact with clinically significant pathogens.

High density lipoprotein cholesterol: HDL-C is known to bind endotoxin, and if given prophylactically lowers cytokine levels and improves survival in animal models of sepsis. In human volunteers given endotoxin, recombinant HDL-C potently reduced the release of TNFα, IL-6, and IL-8, while only modestly attenuating the secretion of proinflammatory cytokine inhibitors IL-1ra, soluble TNF receptors and IL-10 (Pajkrt et al, 1996). No patient outcome trials have yet been published.

Bactericidal permeability increasing protein: BPI is released from neutrophil granules, and has a high affinity for endotoxin (Giroir et al, 1997). In animals it protects against experimental sepsis while in human volunteers it can reduce the cardiovascular changes and cytokine release associated with endotoxin (De Winter et al, 1995). Phase III trials in meningococcal sepsis (395 patients) and trauma (842 patients) have been stopped, but have not yet been reported.

Endotoxin binding columns: One of the most potent endotoxin binding substances is polymixin B, a synthetic antibiotic. Renal toxicity prevents its systemic use, but it can be complexed to immunoglobulins or bound to a haemofiltration circuit. The latter has been associated in a small controlled trial of patients with sepsis and endotoxaemia (n=70) with lower endotoxin and cytokine levels, and improved outcome (54% survival in the treatment group, and 36% in the controls) (Tani et al, 1998). A larger phase II trial is now in progress.

Stop endotoxin's action at the cell surface

Anti-CD14 and LBP monoclonal antibodies: Endotoxin, when bound to LBP, appears to activate the monocyte via cell surface receptors, including CD14 and the toll-like family (*Figure 3*). While monoclonal antibodies against CD14 and LBP reduce cell responses to endotoxin in animal models of sepsis (Schimke et al, 1998), this approach is not yet ready for human trials.

Lipid-A analogues: Although lipid-A is thought to impart most of the pathological effects of endotoxin, there are both natural and synthetically altered forms of lipid-A that are much less pathogenic that can act as lipid-A antagonists. In animals these substances such as monophosphoryl lipid A (MPLA) and E5531 reduce the effects of administered endotoxin and improve survival in experimental sepsis (Chase et al, 1986; Astiz et al, 1995). Phase I trials showed that most of the responses to endotoxin could be abolished or significantly reduced in a dose dependent manner in humans given both endotoxin and the lipid-A antagonist E5531 (Christ et al, 1995). Phase II studies in sepsis are ongoing.

Stop endotoxin's action inside the cell

The sequence of events once endotoxin reaches its target cell (for example the monocyte) is not fully known. Interest has focused on endotoxin

receptors such as CD14 and the toll-like receptor family, which in turn may alter activity in gene promoters such as nuclear factor kappa B (NF-κ B). Therapies aimed at this level are at a preclinical stage.

'Neutralize' products of the target cell

Once endotoxin reaches its 'target cell', be it macrophage, neutrophil or endothelium, it initiates changes such as cytokine production or induction of nitric oxide synthetase. While outside the scope of this article, therapies directed at the products of endotoxin-induced cell activation could in some way be considered 'antiendotoxin therapies'. HM

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KEY POINTS

- Endotoxin can start the processes that lead in the end to organ failure and death during critical illness, and after surgery and trauma.
- Most antiendotoxin substances have failed to show a reduction in mortality in sepsis, and this may be because of the difficulty in intervening once the inflammatory cascade has started when compared to preventing endotoxin-induced inflammatory activation.
- Currently, there is no commercial antiendotoxin agent available for routine clinical practice either in sepsis or for use before situations where we would expect endotoxin release to occur.
- Experimental approaches aimed at stopping endotoxin release involve fluid therapy, antibiotics or lipid-A synthesis inhibitors.
- Free endotoxin may be bound with antiendotoxin antibodies, high density lipoprotein, bactericidal permeability increasing protein or endotoxin binding columns.
- The cellular response to endotoxin may be altered at the level of surface receptors (CD14 and the toll-like receptors) or at an intracellular level.

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