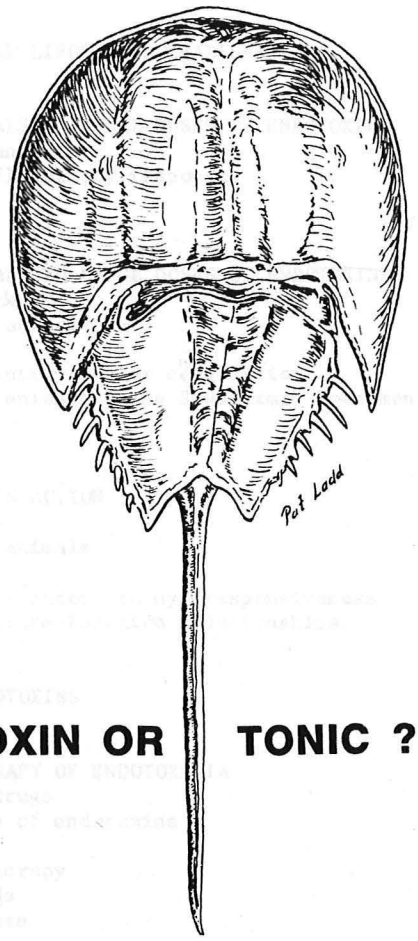
BACTERIAL LIPOPOLYSACCHARIDE



TOXIN OR TONIC ?

MEDICAL GRAND ROUNDS

UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL

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BACTERIAL LIPOPOLYSACCHARIDE: TOXIN OR TONIC?

INTRODUCTION

Endotoxins are bacterial toxins that are intrinsic to the bacterial cell wall, in contrast to exotoxins that are secreted into the surrounding environment. This distinction is actually sometimes confusing, as endotoxins may also be found in outer membrane fragments that are shed from the cell wall into the growth medium.

There is a second important difference between endotoxins and exotoxins. Exotoxins are proteins that often have specific enzymatic activity. For example, diphtheria toxin inhibits protein synthesis by catalyzing the ADP-ribosylation of elongation factor 2 in animal cells. Endotoxins are not enzymes. They are lipopolysaccharides (LPS) that stimulate animal cells to release abnormal amounts of normal compounds. It is these compounds that produce physiologic changes, not the LPS per se.

This presentation will emphasize yet another distinction between bacterial exotoxins and endotoxins. Exotoxins are always toxic. In other words, even in low doses these proteins have no known effects that are beneficial to the susceptible animal host. In contrast, the responses to endotoxins may be beneficial and/or harmful to animals. Small amounts of endotoxin will stimulate the acute inflammatory response, which includes many protective defenses to microbial invasion. In larger amounts, however, endotoxins may induce cells to release an array of compounds so large and so complex that normal homeostasis is disrupted.

The term endotoxin will be used here to refer to the lipopolysaccharides of gram-negative bacteria. This is an accepted usage, although it is recognized that other intrinsic cell constituents, such as peptidoglycan, can produce similar responses in animals. In general, the amount of peptidoglycan that is required to produce a given effect is much larger than the amount of LPS.

For this discussion it is also assumed that lipopolysaccharides stimulate most if not all of the responses of animals to gram-negative bacteria. This is clearly an unproven assumption, yet no other bacterial component so closely reproduces the array of responses seen during gram-negative bacterial infection, and the doses of LPS that provoke significant changes in animals--especially in man--are extremely small.

Finally, I shall focus on the structural and biological features of enterobacterial LPS, ignoring the heterogeneity that exists between the LPS of different gram-negative bacterial families (and indeed within the molecules made by the same bacterium) in an attempt to present a representative overview.

GRAM-NEGATIVE BACTERIAL LIPOPOLYSACCHARIDES

Gram-negative bacterial lipopolysaccharides are complex molecules that have the general structure shown in Figure 1. In enteric bacteria, a polysaccharide (O-antigen) chain is attached via a bridging oligosaccharide core region to a glycolipid that is called lipid A. The O-antigen is highly variable in structure and length; its antigenic diversity is the basis for one important method for serotyping gram-negative bacteria. It has been recognized recently that the LPS of some bacteria do not have an O-antigenic region. These LPS (from Hemophilus, Neisseria, and others) have a relatively short oligosaccharide attached to lipid A; many workers now refer to these molecules as lipooligosaccharides (LOS). This distinction is not important for the following discussion.

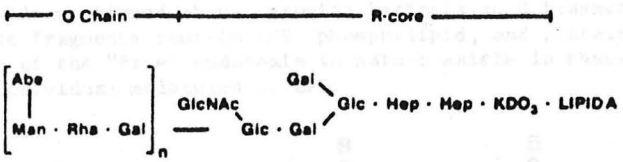


Figure 1. General structure of lipopolysaccharides. The structure of Salmonella typhimurium LPS is shown. KDO = 2-keto-3-deoxy-octulosonic acid; Hep = heptose; Abe = abequose; Rha = rhamnose; GlcNAc = N-acetyl glucosamine.

Lipid A is a phosphorylated glycolipid that is responsible for most of the bioactivities of the LPS. Details of its structure have only recently been established.

LPS are thus complex amphipathic molecules. They can be extracted from the bacterial cell wall with 45% phenol at 68°C (the Westphal procedure) and by a variety of other methods. Purified LPS aggregate with one another to form structures that resemble ribbons or micelles when examined by electron microscopy.

LPS are assembled on the inner (cytoplasmic) membrane of the bacterial cell envelope. They are then irreversibly translocated to the outer membrane, where they are oriented with the hydrophilic polysaccharide region extending into the external environment.

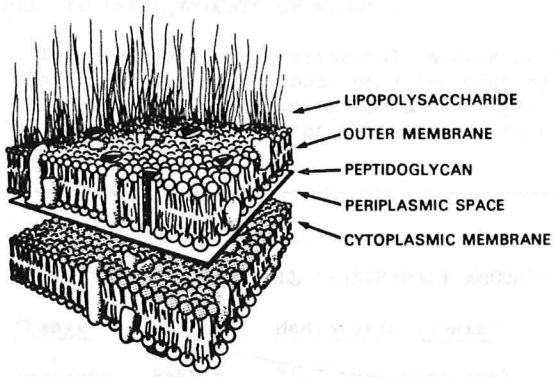


Figure 2. Bacterial cell envelope

As mentioned above, growing bacteria shed fragments of outer membrane. These fragments contain LPS, phospholipid, and protein. It is likely that most of the "free" endotoxin in nature exists in these fragments rather than as individual molecules of LPS.

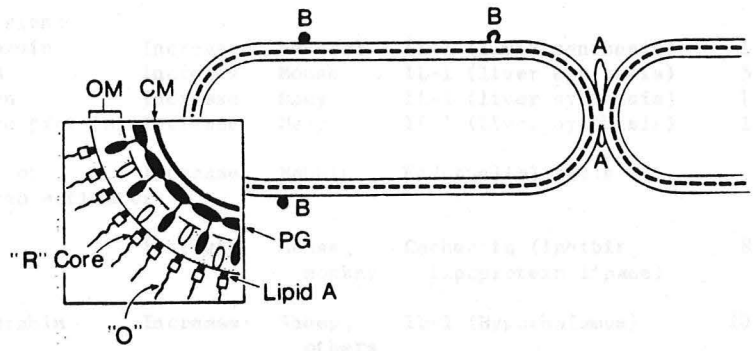


Figure 3. Cartoon showing the location of LPS in the outer membrane (OM), and the shedding of outer membrane fragments during septum formation (A) or from the cell surface (B).
(Copyright. 1978. American Gastroenterological Association. R. S. Munford. Gastroenterology 75:523)

The gastrointestinal tract is a large reservoir of gram-negative bacteria and thus of endotoxin. There is much circumstantial evidence that LPS cross the intestinal wall, enter the portal blood, and are cleared by the liver. It seems likely that these events occur frequently during normal living. In the discussion that follows it will be argued that many of these interactions are beneficial to the animal host. Only when the amount of endotoxin encountered is too large, or perhaps when the method of presentation of the endotoxin changes, do the responses to LPS become harmful.

THE RESPONSES OF ANIMALS TO SMALL AMOUNTS OF ENDOTOXIN

Experimental animals. The responses of experimental animals to LPS have been studied for decades. The bolus injection model used for most of these studies bears little resemblance to the clinical situation, yet the studies have provided valuable information and many of the observed responses have also been noted in human beings.

TABLE 1

RESPONSES OF EXPERIMENTAL ANIMALS TO SMALL (SUBLETHAL) AMOUNTS OF LPS

Parameter	Change	Animal	Mediator/mechanism	Reference
Body temperature	Increase	Rabbit, others	IL-1 (hypothalamus)	1,2
Serum iron	Decrease	Several	IL-1 (Lactoferrin from PMN)	3
Serum zinc	Decrease	Several	IL-1 (? mechanism)	4,5
Blood neutrophils	Decrease, then large increase	Rabbit	Margination, then release of marrow PMNs	
Serum proteins:				
Ceruloplasmin	Increase	Several	IL-1 (liver synthesis)	1,2
Amyloid A	Increase	Mouse	IL-1 (liver synthesis)	5,6
Fibrinogen	Increase	Many	IL-1 (liver synthesis)	1,2
C-reactive protein	Increase	Many	IL-1 (Liver synthesis)	1,2
Inhibitor of Plasminogen activator	Increase	Rabbit	Endothelial cells	7
VLDL	Increase	Mouse, monkey	Cachectin (inhibit lipoprotein lipase)	8,9
ACTH, endorphin	Increase	Sheep, others	IL-1 (Hypothalamus)	10,11

It is now known that many of these effects of endotoxin are mediated by protein hormones that are secreted by endotoxin-stimulated host cells, principally macrophages. One set of these proteins has been called interleukin I (IL-1) (12). Most of the various biological activities of interleukin I were described using materials that were not purified to homogeneity. These activities were initially described as endogenous pyrogen, lymphocyte activating factor, leukocyte endogenous mediator, and others. Various similarities between the activities and physicochemical properties of the molecules led to the "interleukin I" concept. Now that interleukin I molecules have been purified and cloned, it seems likely that more than one protein is involved. For example, one preparation of highly

pure human interleukin I is only weakly pyrogenic (13), yet it has potent activity in the lymphocyte activation assay. It is quite likely that various members of the interleukin I family will have different functions.

One acute-phase protein mediator that is clearly not interleukin I has recently been identified. This is cachectin, a protein (M.W. 17,000) that inhibits lipoprotein lipase (14). It is thought to be responsible for producing the hypertriglyceridemia that occurs in endotoxin-challenged animals. Recent evidence indicates that cachectin acts by inhibiting the synthesis of key lipogenic enzymes, including lipoprotein lipase, in adipocytes (15). Cachectin is identical to another protein, tumor necrosis factor, that has been known for many years to mediate tumor hemorrhage and necrosis when animals with certain tumors were primed with BCG vaccine and then challenged with nonlethal doses of endotoxin (16). Interestingly, unpublished reports suggest that cloned cachectin is much more pyrogenic than cloned IL-1. Cachectin may actually be an endogenous pyrogen!

Note that several of the components of the acute inflammatory response are proteins that are synthesized by the liver. One of these proteins is C-reactive protein (CRP), so named when it was discovered to be a serum protein that binds pneumococcal C-polysaccharide. Blood levels of CRP can increase as much as 2000-fold during acute inflammation, and CRP measurements have even been used to monitor the course of inflammation in patients (it is somewhat analogous to the erythrocyte sedimentation rate, which correlates well with plasma levels of fibrinogen, another acute-phase protein). Although several biological activities of CRP have been known for many years (like immunoglobulins, CRP promotes phagocytosis and complement fixation), a physiological role for CRP has only recently been proposed. CRP binds tightly to chromatin, and it seems likely now that CRP is a scavenger for chromatin fragments that are released from damaged cells. CRP would thus function to remove chromatin from tissue sites (such as local sites of microbial invasion) where cells were damaged or dead (17).

Recent evidence indicates that both cachectin and IL-1 have pre-translational effects on the synthesis of specific proteins. IL-1 increases the levels of mRNA for factor B and serum amyloid A in mouse liver while concomitantly decreasing the level of mRNA for albumin (18). Similarly, cachectin modulates the levels of mRNA for certain genes in adipocytes (15).

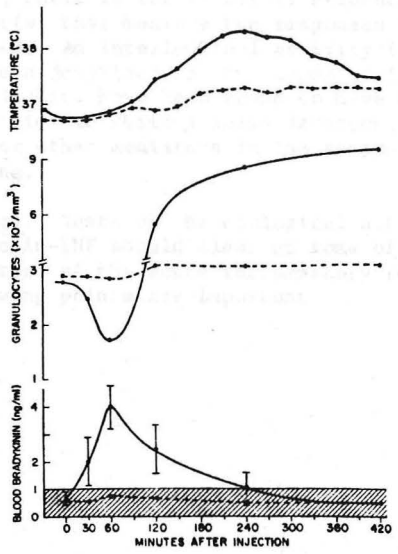
Man. Information about the responses of man to small amounts of LPS comes largely from studies performed in the period 1955-1975, when endotoxins were used as diagnostic agents for evaluating certain hematopoietic and endocrinologic functions. Standard preparations of LPS (Pyromen, Lipexal) were used for most of these studies. One of the important results of these investigations was the realization that man is more sensitive to endotoxic effects than other animal species. Doses of a few nanograms per kg are sufficient to elicit fever and the intravenous administration of a few micrograms will produce shock. Some of the reported effects of low doses of LPS in man are summarized in Table 2.

TABLE 2

RESPONSES OF MAN TO LOW DOSES OF LPS

Effect	Comment	Reference
Fever	Monophasic	19,20
Leukopenia	Not always seen	20,21,25
Leukocytosis	Occurs at sub-pyrogenic doses	19
Thrombocytopenia	Does not involve clotting activation	22
Hypoferremia	Occurs at sub-pyrogenic doses; involves IL-1 release of lactoferrin from PMNs	23,30
Increase in plasma kinin	Occurs before onset of fever and quickly disappears	21
Increase in plasma ACTH and cortisol	Once used to test pituitary-adrenal axis	24

These responses occur with very low doses of LPS (1-10 nanograms/kg body weight). Some of the responses occur at sub-pyrogenic doses. The time-course of some of these events is shown in Figure 4.



Mean temperature, granulocyte and bradykinin responses (± standard error) for four subjects administered intravenous endotoxin (two received 3 mg/kg and two received 5 mg/kg, solid line) or control saline (broken line). Shaded areas for bradykinin levels represents the range for normal values.

Figure 4. Time course of human responses to LPS. (Copyright. 1972. Academic Press, Inc. Kimball HR, et al. Proc Soc Exp Biol Med 139:1080.)

It should be noted that there is little correlation between the degree of leukocytosis and the febrile response (20). Indeed, individuals who have been rendered tolerant to the pyrogenic response to LPS continue to have brisk leukocytosis with each injection (19). Although there is evidence that a serum factor produces the release of granulocytes from the bone marrow (26), the nature of this factor is not known.

There is also evidence that endotoxic effects are mediated indirectly in human beings. One of the responses to sepsis is an increase in muscle proteolysis. Recent studies have shown that (1) in the plasma of febrile patients there is a small MW protein (approximately 4,000 daltons) that has the ability to induce proteolysis in isolated rat muscle (28), and (2) that human interleukin-1 can induce proteolysis of isolated rat muscle (29). It is likely that the former factor is a low-molecular weight fragment of IL-1.

Granulocytes contain a lysosomal protein, lactoferrin, that has great affinity for iron. Human IL-1 induces the granulocytes to release lactoferrin, which then is able to remove iron from circulating transferrin (30). The lactoferrin-iron complex is quickly taken up by the liver, presumably by Kupffer cells. The hypoferremia is thought to be an important host defense mechanism toward microorganisms which require iron for their survival or growth.

Although clinical experience would suggest strongly that low doses of endotoxin also induce the synthesis of acute phase proteins by the human liver, there is little direct evidence for this statement. Similarly, the factor(s) that mediate the responses of man to endotoxin are only poorly defined. An interleukin-1 activity (leukocyte migration-inhibition factor) has been described in the plasma of febrile human patients (27) and human interleukins have been found to have most of the expected biological activities in various animal systems, yet direct evidence for the role of IL-1 or other mediators in the response of man to endotoxin is currently lacking.

Summary. Tests of the biological activities of the cloned interleukins and cachectin-TNF should clear up some of the current confusion about the mediators of the acute inflammatory response. For this discussion, the following points are important:

(1) these in vivo effects of endotoxins appear to be mediated indirectly and to require an interaction between LPS and host cells. The monocyte-macrophage is probably the principal host cell in this interaction, although endothelial cells (167), granulocytes, and others play some role. LPS induces these cells to synthesize specific hormones (IL-1, cachectin, etc), almost certainly by modulating protein synthesis at a pre-translational level (31). The hormones in turn orchestrate the acute inflammatory response, and again the critical step appears to involve pre-translational regulation of protein synthesis in various target organs (liver, adipocytes, etc) (15,18).

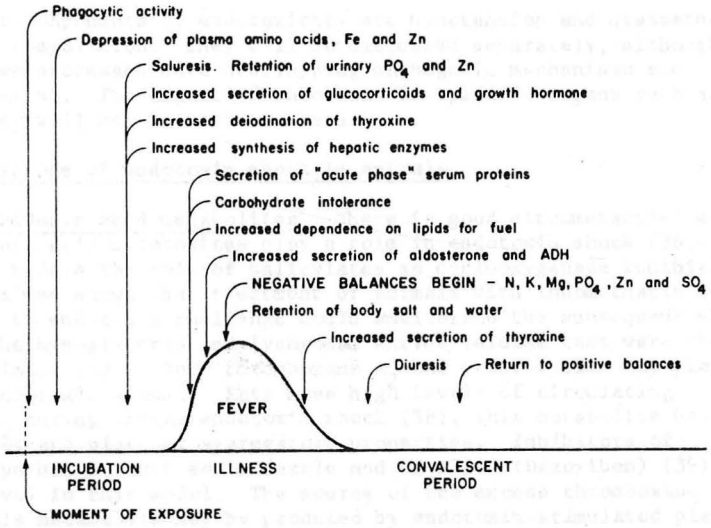
(2) these responses to LPS represent the normal host response to inflammation. Many of the changes would seem to provide some protection to the animal host from invading microbes or to assist in tissue repair.

TABLE 3

ENDOTOXIN-INDUCED RESPONSES--POSSIBLE BENEFITS TO THE HOST

Response	Possible benefit
Fever	Slow growth of invading organisms (32)
Leukocytosis	Mobilize neutrophils to site of infection
Low serum iron and zinc	Slow microbial growth (33)
C-reactive protein	Scavenge chromatin from tissues (17)
High ceruloplasmin	Protect host copper (needed for phagocyte superoxide dismutase, cytochromes)
ACTH release	Stress response--cortisol increases
Muscle catabolism	Provide amino acids for protein synthesis (34)
Inhibit lipoprotein lipase	Shift to lipid catabolism

(3) these responses are not specific for LPS. In other words, other stimuli may produce similar effects, presumably by stimulating macrophages and other cells to release the same mediators (1). A graphic summary of the acute inflammatory sequence has been provided by Beisel (34):



Schematic representation of the sequence of nutritional responses that evolves during the course of a "typical" generalized febrile infectious illness (5).

Figure 5. Acute inflammatory response
(Copyright. 1977. American Society for Clinical Nutrition. W.R. Beisel. Am J Clin Nutr 30:1236.)

(4) these responses to inflammatory stimuli are very primitive--the horseshoe crab (Limulus polyphemus), when challenged with endotoxin, produces an interleukin-1 like molecule that increases C-reactive protein synthesis. The horseshoe crab evolved some 200-400 million years before man (35). The acute inflammatory response to endotoxin has thus been highly conserved!

(5) prolonged stimulation of the acute inflammatory reponse, such as with chronic infection, can lead to deleterious effects such as muscle wasting, weight loss, etc.

RESPONSES OF ANIMALS TO LARGE AMOUNTS OF ENDOTOXIN

There are striking differences in the susceptibility of various animals to endotoxin lethality, and unfortunately man is more sensitive to endotoxic responses than any commonly used experimental animal (with the possible

exception of the rabbit). There are also species differences in the pathologic responses to endotoxin. Although extrapolation of the results of animal studies to the clinical situation in man would thus seem hazardous, the animal studies are important for possible clues to pathogenesis and effective therapy.

Two major components of endotoxicity are hypotension and disseminated intravascular coagulation. They will be discussed separately, although clearly the two processes have overlapping pathogenic mechanisms and frequently coexist. The impact of endotoxin on specific organs such as the lung and kidney will not be reviewed here.

Potential mediators of endotoxin shock in animals.

(1) Arachidonic acid metabolites. There is good circumstantial evidence that arachidonic acid metabolites play a role in endotoxin shock (36,44a). Several years before the role of salicylates as cyclooxygenase inhibitors was discovered, it was shown that treatment of animals with indomethacin or aspirin prior to endotoxin challenge could ameliorate the subsequent shock and prevent the hypoglycemia and lysosomal enzyme release that were observed in control animals (37). Both thromboxane A_2 and prostacyclin may play a role in the endotoxic animal. Rats have high levels of circulating thromboxane A_2 during lethal endotoxin shock (38); this metabolite has potent vasoconstrictor and platelet aggregatory properties. Inhibitors of thromboxane synthesis, such as imidazole and UK 37248 (Dazoxiben) (39), prolong survival in this model. The source of the excess thromboxane A_2 is not known; this metabolite may be produced by endotoxin-stimulated platelets, neutrophils, or macrophages.

In primates and sheep, the peak in thromboxane A_2 levels occurs early after endotoxin administration, at a time when the pulmonary artery pressure and altered pulmonary mechanics are maximal (41). In contrast, prostacyclin levels seem to correlate inversely with the arterial blood pressure, rising as systemic hypotension develops. The role of prostacyclin in producing the hypotension is uncertain, as in other studies the infusion of this metabolite, which has vasodilatory activity and inhibits platelet aggregation, appears to increase survival in canine endotoxin shock (42).

Leukotrienes are products of the lipooxygenase pathway. Leukotrienes C_4 and D_4 produce plasma leakage from postcapillary venules, while leukotriene B_4 promotes adhesion of leukocytes to capillary endothelium and the release of neutrophil lysosome contents (43). Inhibition of leukotriene synthesis has been reported to ameliorate endotoxin shock in the rat (44); the inhibitors lack sufficient specificity to allow definite conclusions (44a).

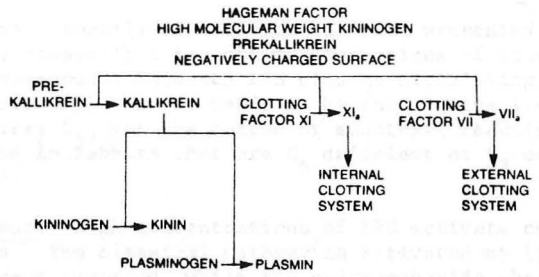
Interestingly, arachidonic acid infusion protected endotoxin-challenged rabbits from death: the protection was abolished when sodium meclofenamate, a prostaglandin synthetase inhibitor, was also given (45).

Numerous nonsteroidal antiinflammatory agents have been studied in various models of endotoxin shock. In general, (1) pretreatment of animals

with these agents before the injection of endotoxin is associated with prolongation of survival, (2) treatment of animals after the endotoxin injection is ineffective, and (3) the drugs probably have important effects in addition to inhibition of cyclooxygenase (and, in high dose, lipooxygenase) enzymes (44a). It is important to note that these agents do not block all of the cellular responses to LPS--doses that prevent fever have no effect on the hypoferremia and hypozincemia that occur after endotoxin administration (4, 5, 46), nor do they prevent endotoxin-induced leukocytosis or changes in hepatic protein synthesis (5,46) or amino acid metabolism (46).

(2) Platelet activating factor (PAF). 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (PAF) is an extremely potent inducer of platelet aggregation, hypotension, and bronchoconstriction. Although interest in this compound has principally concerned its possible role as the mediator of IgE-induced anaphylaxis (47), there is also evidence that it may figure in the pathogenesis of endotoxin shock. A synthetic compound that inhibits the effects of PAF, CV-938, has been reported to prevent endotoxin-induced shock in rats and to protect against endotoxin lethality. Interestingly, the drug could also reverse established endotoxin-induced hypotension (48). Large doses of CV-938 were required to achieve these effects; little is known about the toxicity or other effects of the compound.

(3) Bradykinin. Activation of the kinin and clotting systems clearly occurs in animals that receive large doses of LPS. Indeed, there is much evidence that kinins contribute to endotoxin shock in both animals and man.



The sequences of activation of components of the Hageman factor system.

Figure 6.
(Copyright. 1985. Elsevier Science Publishers. Cochrane CG. Handbook of endotoxin, Vol. 2. Pathophysiology of endotoxin, 286-298.)

Kinin formation is thought to occur when the Hageman factor (XII)-high molecular weight kininogen-prekallikrein-Factor XI complex is activated by the lipid A region of LPS (50), leading to cleavage of bradykinin from high molecular weight kininogen. The complex relationships between these plasma proteins and the proteins of the clotting, fibrinolytic, and complement cascades are understood in considerable detail (see review, ref. 51). The

lack of specific inhibitors of bradykinin, which has vasodilatory and vascular permeability-increasing properties, has impeded careful definition of the role of this mediator in endotoxin shock.

(4) Endogenous opiates (endorphins). β-endorphin is a 31 amino acid peptide that arises from the same precursor molecule that contains ACTH. In response to stress, ACTH and β-endorphin are released in equimolar amounts, and dexamethasone administration suppresses the secretion of both molecules. Opiates have been known for years to have hypotensive effects. Interest in a role for endorphins in the pathogenesis of endotoxic shock was sparked by the report of Holaday and Faden that high doses of naloxone (10 mg/kg) could reverse endotoxin-induced shock in rats (52). Subsequent evaluation by many workers has established that (a) high doses of naloxone are required for the anti-hypotensive effect--the necessary naloxone concentrations are much higher than are necessary to saturate opiate receptors, suggesting that some other activity of the drug may be operative, (b) there is stereospecificity to the effect, as only the levorotatory stereoisomer of naloxone is effective, (c) the naloxone effect is also seen in hemorrhagic and spinal shock, (d) increased β-endorphin levels have been found in animals with endotoxin shock, and (e) there is cross-tolerance with morphine (reviewed in 53). Studies in rats, pigs, horses, and dogs have supported the initial claims. Measurements of β-endorphin levels in endotoxin-challenged sheep found that there were two peaks of β-endorphin release, one occurring early (before the onset of fever) and the other late (along with the fever) (11). Naloxone actually increased the amounts of β-endorphin in both peaks, suggesting that there is normally feed-back inhibition of β-endorphin release.

(5) Platelets. Compelling evidence has been presented that platelets are not primarily responsible for the manifestations of endotoxic shock in the rabbit. Intravenously injected LPS bind to circulating platelets, apparently by a reaction that is mediated by the alternative complement pathway and requires C_3, yet the course of endotoxic reactions is the same in normal rabbits and in rabbits that are C_6 deficient or C_3-depleted with cobra venom factor (54).

(6) Complement. High concentrations of LPS activate complement in vitro by two mechanisms. The classical pathway is activated by lipid A in an antibody-independent reaction, while the polysaccharide chain is capable of activating the alternative pathway (55). Recent evidence indicates that only the alternative pathway is activated by native LPS in vitro; in vivo, activation of the alternative pathway also predominates. As indicated above, there is little convincing evidence that complement activation mediates the toxic effects of LPS in vivo.

TABLE 4

PHARMACOLOGICAL INTERVENTIONS THAT REVERSE ENDOTOXIN SHOCK OR LETHALITY

Intervention	Proposed Mechanism of Action	Species	Effect on Shock	Effect on Lethality	Ref.
Aspirin Indomethacin	Cyclooxygenase inhibitors	Dog, rat	yes	yes	37,40
Imidazole UK 37248	Thromboxane synthetase inhibitors	Rat	-	yes	36,39
Diethylcarbamazine	Leukotriene synthetase Inhibitor	Mouse	-	yes	44,44a
Naloxone	β-Endorphin inhibitor	several	yes	yes	52,53
CV-938	PAF antagonist	Rat	yes	yes	48
Trasylol	Serine protease inhibitor	Dog	no	yes	56
Fructose-1-6-diphosphate	Prevent hypoglycemia	Dog	yes	yes	57
Glucocorticoids	Multiple	many	yes	yes	--
Antibodies to cachectin	Uncertain	Mouse	-	yes	58

It is apparent that multiple agents, which presumably act via independent mechanisms, can reverse endotoxic shock in animals. Why do such different agents reverse endotoxic shock? It would appear that hypotension is the product of many interactions, some of which are additive or synergistic. Remove one of the contributing factors and there is at least transient improvement. There is no single mediator of endotoxic shock. In different animal species, and probably in different human patients, the array of interacting factors may be different.

Potential mediators of endotoxic shock in man.

Gram-negative bacteremia: the clinical presentation. The earliest detectable physiologic alteration in patients with gram-negative bacteremia is hyperventilation. This is followed by fever, leukopenia and/or leukocytosis, hypotension, and metabolic (lactic) acidosis. Bacteremic shock classically develops in two phases. First, the "warm" phase characterized by decreased systemic vascular resistance (SVR), increased cardiac output, venous pooling of blood, normal or increased central venous pressure, and warm extremities. During this hyperdynamic phase the patient is often alert, hyperventilating, with bounding pulses. The second phase

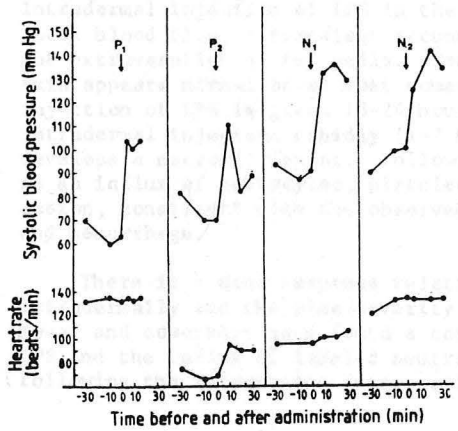
more closely resembles classical shock, in which the patient is obtunded, with cold clammy skin and a rapid but thready pulse. The hemodynamic picture in this "cold" phase includes increased peripheral resistance, decreased central venous pressure, and often a failing myocardium (59).

Gram-negative bacteremic shock: possible mediators. Relatively few of the alleged mediators of endotoxic shock in animals have been evaluated in clinical shock. Moreover, the available studies consist of measurements made before or during shock; a small number of parameters is usually measured and there has been little opportunity for intervention with specific inhibitors.

(1) Bradykinin. In man, low doses of endotoxin produce an increase in plasma kinin level, as noted above. The increase in kinins, moreover, is a very early response to LPS injection, occurring before the onset of fever or leukocytosis (21). Activation of the kinin system has also been documented in several studies of clinical sepsis (59), and the administration of bradykinin to man is followed by vasodilatation. Bradykinin thus is a strong contender for a role as a hypotensive agent in human bacteremic shock.

(2) Thromboxane A_2. A single study has reported that human patients with septic shock had elevated plasma thromboxane A_2 levels (60).

(3) Endogenous opiates. Great interest in the potential clinical use of naloxone, a morphine antagonist, followed the report of Faden and Holladay in 1978 that naloxone could reverse experimental endotoxic shock (52). Numerous anecdotal reports have claimed that naloxone can increase the blood pressure in human patients with septic shock (61). A recent randomized trial of low dose (0.4 - 1.2 mg) bolus naloxone therapy in septic shock failed to show a difference between naloxone and placebo. Moreover, some of the patients had dramatic responses to the injection of placebo, as is shown in the Figure from DeMaria et al (61). A clinical trial using higher doses of naloxone is in progress.



—Systolic blood pressure and heart rate before and after the administration of one vial of placebo in two subjects (P_1, P_2) and one vial of naloxone (0·4 mg) in two subjects (N_1, N_2).

Data are presented for those subjects who required only one vial of naloxone or placebo.

Figure 7. Naloxone trial
(Copyright. 1985. The Lancet Ltd. DeMaria A, et al. Lancet 1:1363-1364.)

(4) Complement-related anaphylatoxins. Activation of the alternative pathway of complement has been documented in patients with gram-negative bacteremia; interestingly, only those patients who subsequently developed shock had complement factor activation in the specimen obtained at the time of blood culture (62). There are at least two potential anaphylatoxins that might be produced during complement activation.

It should be emphasized that in both man and experimental animals there is a dynamic equilibrium between activators and inhibitors of the various proteolytic cascades. An increase in C1 esterase inhibitor, which opposes the activation of Hageman factor (and therefore of kallikrein), has been documented in bacteremic patients (63); presumably the concentration of this inhibitor increases as part of the acute inflammatory response. Inhibitors of the contact activation system would appear to dampen the endotoxin response and thus to be beneficial. In any case, it is extremely difficult to be sure that an increase or decrease in the factor measured is a primary event and not a compensatory change in response to another alteration.

Studies of septic shock in man have focused largely on the plasma serine proteases and their products. As in animals, the process probably involves a complex array of factors, and there is circumstantial evidence that leukocytes may play a role in human shock: neutropenic patients may have a reduced risk of shock during gram-negative bacteremia (64,65).

Disseminated Intravascular Coagulation.

Disseminated intravascular coagulation (DIC) is a well-known feature of endotoxin administration to animals. Characteristic changes include decreases in Factor II, V, VIII, and fibrinogen levels, with increased levels of fibrin split products. Thrombocytopenia is common.

DIC in animals: the local and generalized Shwartzman phenomena. Intradermal injection of LPS in the rabbit is followed by an increase in local blood flow, a transient accumulation of neutrophils and platelets, and the extravasation of red cells. Despite these local changes, the area of skin appears normal or at most somewhat erythematous. If an intravenous injection of LPS is given 18-24 hours later, however, the site of the intradermal injection rapidly (1-2 hours) becomes hemorrhagic and eventually develops a necrotic center. Following the provocative injection of LPS there is an influx of leukocytes, platelets, fibrinogen, and red cells into the lesion, consistent with the observed histopathological changes of thrombosis and hemorrhage.

There is a dose-response relationship between the amount of LPS injected intradermally and the size/severity of the lesion that forms. Similarly, Movat and coworkers have found a correlation between the intradermal dose of LPS and the influx of labeled neutrophils and platelets into the lesion following the intravenous (provocative) injection:

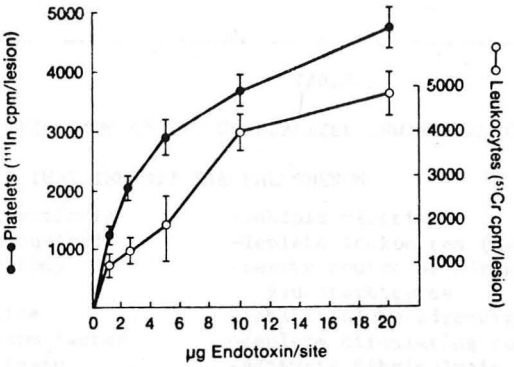


Figure 8. (Copyright. 1981. Elsevier Science Publishers. Movat HZ. Handbook of Physiology. The cardiovascular system IV. p 1047.)

It has been almost 6 decades since this interesting phenomenon was described by Shwartzman (66). Despite much interest and research the pathogenesis of the phenomenon remains unknown. It is thought that the preparative injection of LPS induces chemotaxis of leukocytes into the lesion, that the LPS localize in these leukocytes after the intradermal injection, and that the leukocytes may produce procoagulant substances and proteases that initiate the local thrombohemorrhagic reaction (67,68). The effect of the intravenous dose is uncertain, although much more is known about it (see below). There are several clues to the genesis of the local Shwartzman phenomenon: it is blocked by heparin, suggesting a prominent role for activation of the clotting system; it does not occur in animals that are leukopenic as the result of nitrogen mustard administration; and it is not produced by lipid A molecules that lack acyloxyacyl fatty acids or the C-1 or C-4' phosphates.

The priming dose of LPS does not have to be intradermal. Injection of LPS into a renal artery followed by an intravenous injection of LPS produced a local thrombohemorrhagic reaction in the injected kidney, while the other kidney remained normal (69).

The generalized Sanarelli-Shwartzman phenomenon. It has also been known for many years that two sequential doses of endotoxin can produce a dramatic alteration in animal physiology. Although Shwartzman did not describe a generalized reaction, the phenomenon has often been erroneously called the "generalized Shwartzman phenomenon." If the preparative dose is given intravenously and a second (provocative) intravenous dose is given 24 hours later, the animal rapidly develops intravascular coagulation with fibrin deposition in the blood vessels of the kidneys and other organs. Renal cortical necrosis from glomerular capillary thrombosis is the hallmark lesion, but leukocyte-platelet thrombi are found in other organs. The disseminated intravascular coagulation and hypotension are usually rapidly fatal.

TABLE 5

MODIFICATION OF THE GENERALIZED SHWARTZMAN PHENOMENON (70)

A. MANEUVERS THAT INHIBIT THE PHENOMENON

Heparin, warfarin	-inhibit clotting
Nitrogen mustard	-deplete leukocytes (neutrophils, monocytes)
Adrenalectomy	-remove source of adrenal catecholamines, glucocorticoids
Dibenzyline	-inhibit alpha-adrenergic receptors
Cobra venom factor	-deplete circulating complement
Streptokinase	-activate fibrinolysis

B. MANEUVERS THAT PRODUCE THE SAME PATHOLOGY WITHOUT LPS INJECTION

Thrombin infusion plus epsilon aminocaproic acid (inhibit fibrinolysis)
Thromboplastin infusion
Infusion of purified Factor XII plus norepinephrine into pregnant rats (pregnancy is associated with impaired fibrinolysis)

C. MANEUVERS THAT SUBSTITUTE FOR THE FIRST DOSE OF LPS

Reticuloendothelial blockade with thorotrast or trypan blue
Epsilon aminocaproic acid infusion (inhibit fibrinolysis)
Pregnancy (decreased fibrinolysis?)
Cortisone (? increase sensitivity of the microcirculation to alpha-adrenergic stimuli)
Infusion of acidic polymers (dextran sulfate, others)

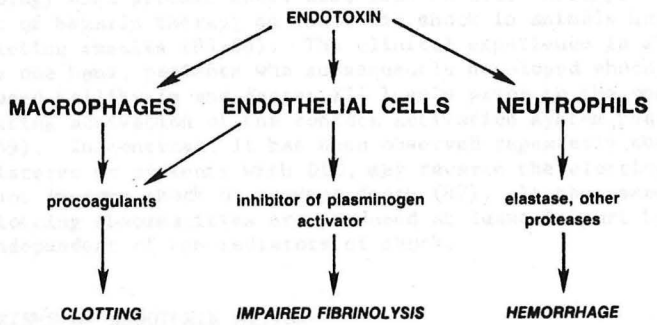
Lewis Thomas proposed, to account for the generalized phenomenon, that the preparative dose of LPS mobilizes granulocytes, which produce (directly or indirectly) a substance that precipitates fibrinogen and produces the "fibrinoid" that is found in the blood vessels that are involved in the reaction. Dextran sulfate and other acidic polymers would substitute for the preparative injection by providing a substitute for the putative fibrin-precipitating substance (71).

McKay developed this hypothesis into a more general synthesis (70). He argued that the generalized Shwartzman reaction is the result of a complex interplay between leukocytes, platelets, complement, the contact system of plasma (Factor XII and others), adrenal glucocorticoids and catecholamines, alpha-adrenergic receptor sites, and the fibrinolytic system. The initial dose of LPS would prepare for the phenomenon by decreasing the removal of clotting factors (via RES blockade) and by decreasing fibrinolysis. Leukocytes would play a prominent role by producing procoagulants and releasing lysosomal enzymes. Adrenal glucocorticoids would increase the sensitivity of arterioles to catecholamines. High fibrinogen levels, stimulated as part of the acute phase response, would provide ample substrate for intravascular clotting after the second injection. Although this hypothesis is clearly speculative, it takes into account most of the observations cited in Table 5.

Most workers agree that the generalized Shwartzman phenomenon represents a form of DIC. There has been much evidence that leukocytes are essential for the phenomenon, although there is disagreement about the relative importance of monocyte-macrophages and neutrophils for producing it. Although both the local and generalized Shwartzman reactions do not occur in animals that have been made leukopenic by agents such as busulfan and nitrogen mustard, these drugs probably have effects on monocyte-macrophages as well as neutrophils. Replacement experiments using exudate cells have also generally assumed that the cells were neutrophils, yet heterogenous mixtures of leukocytes were probably used (67).

The case for the neutrophil has been summarized by Horn (72). Most recent work has tended to implicate the macrophage as the most likely source of the critical procoagulant factor(s) that initiate thrombosis. For example, various cell-surface procoagulants have been described in human monocytes (73), endotoxin-stimulated hepatic macrophages (74), and peritoneal macrophages (75). Moreover, as will be discussed below, it seems likely that monocyte-macrophages are the principal cellular target for endotoxin that is injected into animals. Other cells may also play roles; a cell-surface procoagulant has been described in endotoxin-stimulated endothelial cells (76,77), and these cells are also thought to be the source of an inhibitor of plasminogen activator (thus, an inhibitor of fibrinolysis) that has been demonstrated in the blood of rabbits injected with low doses of LPS (7). On the other hand, there is substantial evidence that platelets are not critical for the development of endotoxin shock or DIC (78).

These recent observations are generally consistent with the proposal of Muller-Berghaus that endotoxin activates DIC by (1) initiating intravascular coagulation (such as via cell-surface procoagulants), so that soluble fibrin is generated, (2) precipitation of fibrin monomers to form clots, and (3) inhibition of fibrinolysis (79). Macrophages and endothelial cells appear to be the critical cells for initiating and perpetuating thrombosis, while neutrophils may contribute to hemorrhage by releasing oxidants and proteases (including elastase) that disrupt vascular integrity (67).



It should be noted that several modern factors, such as arachidonic acid metabolites and platelet-activating factor, have not been studied carefully in endotoxin-induced DIC. It would be very surprising, given the apparent roles of these compounds in endotoxin shock, if they did not contribute in

some way to the Shwartzman phenomena. Indeed, a recent report indicates that prostacyclin infusion can prevent the generalized Shwartzman reaction induced by LPS in pregnant rats (80).

In both the local and generalized Shwartzman phenomena, the timing of the two doses of LPS is critical. An interval of 18 to 24 hours between the priming and provocative doses is optimal. Moreover, the provocative dose that is required to produce the generalized reaction is usually lower than the dose required to produce shock or DIC in animals that have not been so primed.

Disseminated intravascular coagulation in man. Although gram-negative bacteremia may be one of the most common causes of DIC in clinical practice, only a small fraction of patients with gram-negative bacteremia develops DIC. Often these patients are also hypotensive. A much higher percentage (about 60%) of patients with gram-negative bacteremia has thrombocytopenia, possibly produced by a different mechanism than the DIC; anti-platelet antibodies have been proposed as a possible mechanism (81).

In fulminant meningococcemia, profound hypotension is accompanied by DIC and cutaneous hemorrhage. In epidemics of meningococcal disease, when the same strain of N. meningitidis is presumably responsible for all of the cases, only 10-15% of the patients will develop the fulminant form of meningococcal disease (the rest survive long enough to get meningitis, which has a much better prognosis). The host factors that predispose to the development of the fulminant form are not known. This entity may be the human counterpart of the generalized and local Shwartzman phenomena; meningococci can be found in the hemorrhagic skin lesions, where it is hypothesized that they "prepare" the skin for the phenomena "provoked" by continuing endotoxemia (82). Gram-negative bacteremia during pregnancy can also be associated with renal cortical necrosis, suggesting a Shwartzman-like pathogenesis (83).

It is not known with certainty whether the same derangements in host physiology that produce shock also lead to DIC. Attempts to evaluate the effect of heparin therapy on endotoxic shock in animals have given conflicting results (84-86). The clinical experience is also inconsistent. On the one hand, patients who subsequently developed shock were found to have decreased kallikrein and Factor XII levels prior to the onset of shock, suggesting activation of the contact activation system that might lead to DIC (59). In contrast, it has been observed repeatedly that heparin therapy, administered to patients with DIC, may reverse the clotting abnormalities but does not improve shock or prevent death (87). It thus seems possible that the clotting abnormalities are produced at least in part by mechanisms that are independent of the mediators of shock.

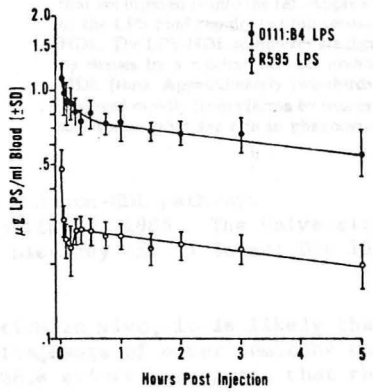
MECHANISMS OF ENDOTOXIN ACTION

The mechanisms by which endotoxin stimulates animal cells are not known. This discussion will review recent results in three areas: (1) the fate of LPS in vivo, (2) the evidence for a critical gene product, (3) structure-function relationships of lipid A.

ENDOTOXEMIA

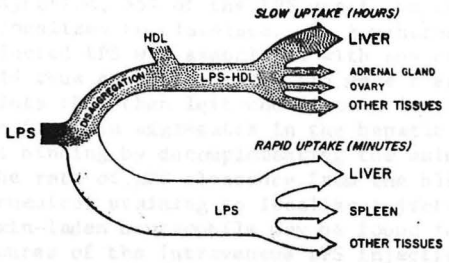
Studies in experimental animals.

Purified radiolabeled LPS that are injected intravenously into experimental animals leave the blood in two phases (88). Some of the LPS are rapidly removed from the blood, apparently either because they bind to blood cells (neutrophils, monocytes, platelets) that then leave the circulation or because they are removed by certain tissues, such as the liver and spleen, that are rich in phagocytic cells. Other LPS quickly bind to circulating high density lipoproteins (HDL) and then remain in the blood for hours, slowly undergoing uptake by tissues that utilize the HDL (89).



The clearance of LPS from the blood of rabbits after a 250-μg i.v. injection of either O111:B4 or R595 LPS. Initial blood LPS concentrations of 1.5 to 2 μg/ml would be expected on the basis of the dose of LPS administered and the blood volumes (125 to 166 ml). In blood samples taken 5 and 180 min post-injection, greater than 95% of the radioactivity was contained in plasma. Each curve represents data from six animals.

Figure 9. Disappearance of radiolabeled LPS from blood (IV injection into rabbits).
 Mathison JC, Ulevitch RJ. J Immunol 123:2133-2143).



Schematic diagram to show the fate of LPS that are injected iv into the rat. Approximately one-third of the LPS bind rapidly (within seconds to minutes) to HDL. The LPS-HDL complexes are then taken up slowly by tissues by a mechanism that involves receptors for HDL (*top*). Approximately two-thirds of the LPS are removed rapidly from plasma by tissues, such as the liver and spleen, that are rich in phagocytic cells (*bottom*).

Figure 10. HDL and non-HDL pathways
(Copyright. 1985. The University of Chicago Press. Munford RS, Dietschy JM. J Infect Dis 152:177-184.

During infection in vivo, it is likely that LPS exist in bacterial carcasses or in fragments of outer membrane that are shed as the bacteria grow. The available evidence suggests that these LPS can also bind to HDL -- in fact, phenol-extracted LPS seem to mimic the fate of native LPS in membrane fragments rather well (90).

These events may be dissected into several steps:

1. Disaggregation of the LPS. The evidence that LPS are disaggregated in blood consists of in vitro observations that LPS disaggregate before they bind to HDL (88,91). Moreover, both anti-LPS antibodies (92) and an acute phase protein (93) appear to prevent LPS-HDL binding by inhibiting the disaggregation of the LPS. The factors in plasma that promote LPS disaggregation are not known.

The native LPS that are in bacterial outer membrane fragments also require some preparative step, presumably dissociation from the membrane, before they will bind to HDL (90).

2. Binding of LPS to blood cells. Estimates of the percentage of an injected bolus of LPS that binds to circulating blood cells are difficult to derive. These estimates are hazardous because the cells that bind LPS may be rapidly removed from the blood so that they are not easily counted, and the various LPS preparations may differ in their ability to bind to cells (78,89). Moreover, some of the reported observations seem conflicting. Mathison and Ulevitch injected radiolabeled LPS intravenously into rabbits

and followed the label over time (78,89). In blood samples obtained in the first two minutes after injection, 35% of the LPS was found in the buffy coat and 86% of the label was localized to platelets. By 5 minutes after injection, none of the injected LPS was associated with any cellular blood element. Although it would thus appear that the LPS were cleared from the blood by binding to platelets that then left the circulation (indeed, LPS-labeled platelets were found in aggregates in the hepatic sinusoids), inhibition of LPS-platelet binding by decomplementing the animals had no discernable effect upon the rate of LPS clearance from the blood (94). Other studies, using immunocytochemical staining to localize injected LPS in the rat, revealed that endotoxin-laden neutrophils may be found in the liver sinusoids within a few minutes of the intravenous LPS injection (95); both neutrophils and platelets that have bound LPS may be subsequently phagocytosed by liver macrophages (Kupffer cells) (89,95). Monocytes carrying LPS have also been found in the walls of blood vessels, apparently working their way through the vessel wall (96). Most authors agree that monocytes are the most important LPS-binding cell.

3. Binding of LPS to HDL and tissue uptake of LPS-HDL complexes. Approximately one-third of an injected dose of LPS rapidly (seconds to minutes) binds to HDL. These LPS then essentially ride piggyback on the HDL, undergoing uptake by tissues that utilize the HDL-cholesterol for various purposes, including sterol synthesis. The bulk of the HDL-bound LPS is taken up by the liver, with a half-life of several hours. Interestingly, LPS-HDL complexes appear to accumulate preferentially in the adrenal glands; although the fraction of the total dose that ends up in the adrenal glands is low (less than 2%), on a weight basis (ug LPS/mg tissue) the uptake by the adrenal glands is similar to that by the liver. Adrenal uptake of LPS-HDL complexes is under hormonal control (pretreatment with ACTH increases uptake, pretreatment with dexamethasone decreases it); as would also be expected if uptake were governed by the uptake of the HDL, high levels of circulating HDL suppress LPS-HDL uptake (97).

4. Uptake of LPS by tissue phagocytes. Approximately two-thirds of an injected dose of LPS is removed rapidly from the blood into the liver, lungs, spleen, and other tissues. It is likely that this tissue uptake is both direct (i.e., via binding of LPS to specific cells within the tissues, such as Kupffer cells in the liver (98)) and indirect (trapping of LPS-loaded blood cells).

5. Degradation of LPS. Little is known about the metabolism of LPS by animals. When radiolabeled LPS are injected intravenously into rats, after two weeks approximately 30 per cent of the injected label appears in the stool (approximately 2% per day) and 7 per cent in the urine. It appears that the LPS in the liver undergo deacylation of the lipid A moiety as well as degradation of the O-antigen (99); nothing is known about the metabolic processes involved. As will be discussed below, neutrophils and macrophages contain enzymes that deacylate lipid A. The role of these enzymes in vivo is also uncertain.

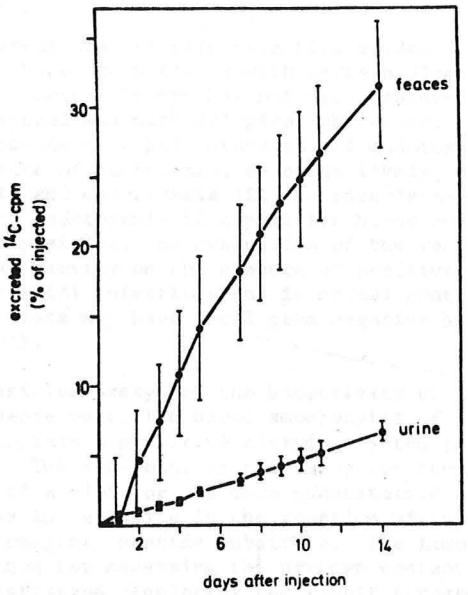


Figure 11. Appearance of radiolabeled LPS in the urine and stool. (Copyright. 1984. Verlag Chemie. Freudenberg MA, et al. Bacterial endotoxin, 295-304.

Factors that modulate the fate of LPS in vivo:

1. Disaggregation -- inhibited by antibody to LPS (92) and by an acute-phase protein (93)

2. LPS-HDL binding -- blocked if LPS are not disaggregated; possibly increased when hypocalcemia is present.

3. Uptake of LPS-HDL complexes -- inhibited by excess HDL or by pretreatment with dexamethasone; increased by pretreatment with ACTH. IgG antibodies to LPS promote uptake of LPS-HDL complexes by tissues that take up immune complexes (liver, spleen) (92).

4. Uptake of LPS that are not bound to HDL (presumably, in aggregates or attached to blood cells) -- enhanced by anti-LPS antibodies, also by dexamethasone pretreatment. Stimulation of the reticuloendothelial system promotes LPS uptake, while RES blockade decreases it (100).

Endotoxemia in man.

Although a great deal of circumstantial evidence suggests that LPS may be present in the blood of patients with certain diseases, definitive proof that "endotoxemia" occurs in man has not been achieved. There are significant conceptual and methodological obstacles. The major conceptual problem is the absence of a gold standard: if endotoxin can appear in the blood in the absence of bacteremia, as seems likely, then the correlation between bacteremia and endotoxemia will be imperfect--there will be false positive tests for endotoxemia if a positive blood culture is required for a test to be a true positive. So evaluation of the results of tests for endotoxemia relies heavily on the absence of positive tests in patients with gram-positive bacterial infections and in normal controls; patients with "false positive" tests may have local gram-negative bacterial infection or liver disease (101).

The most sensitive assay for the bioactivity of LPS (actually, lipid A) is the Limulus lysate test. The blood amoebocytes of Limulus polyphemus, the horseshoe crab, contain a primitive clotting system that is exquisitely sensitive to LPS. The end-point of the assay for the presence of endotoxin is the formation of a clot, or, in more quantitative adaptations of the assay, an increase in turbidity in the reaction mixture or an increase in the cleavage of a chromagenic peptide substrate. The Limulus lysate assay is now an acceptable method for assessing the pyrogen content of fluids for parenteral administration, replacing the rabbit pyrogen test that has traditionally been used for this purpose.

Clinically, the Limulus lysate test has been most successful for the diagnosis of gram-negative bacterial meningitis, achieving a sensitivity of nearly 100% with high specificity (102). When used to detect LPS in plasma, the test has been much less reliable. There are several problems with endotoxin assays in plasma. First, there are inhibitors of the Limulus lysate assay that must be removed before plasma samples can be assayed (103). Second, there is much variability between individual plasma samples; plasma samples from different individuals that are spiked with LPS give variable recovery of the LPS when assayed (104). Third, there are questions about the specificity of the test when used in plasma, as serine proteases are known to produce clotting in the Limulus system. No study of endotoxemia has used immunoadsorption of the LPS to evaluate the specificity of the Limulus assay. Fourth, quantitative measurements of plasma endotoxin levels could be clinically useful, yet comparisons of the results from samples obtained from different patients (with presumably different endotoxins), using a single purified LPS assay standard, may be misleading. Serial measurements in the same patient may be more valuable. Finally, as noted above, there is the problem that endotoxemia may exist without bacteremia, yet strict proof that this occurs will require confirmation by an independent assay.

In spite of these misgivings, there is a large literature on the use of the Limulus lysate test to detect endotoxemia in man. One of the more promising studies is that by workers in Amsterdam (105). In a prospective study they measured endotoxin levels in the plasma of patients whose blood samples were submitted simultaneously for culture. The results are summarized in Table 6.

TABLE 6

Plasma Endotoxin Assays (Limulus test) in Patients with Suspected Sepsis

	Blood culture for gram-negative aerobes	
Limulus result:	positive	negative
positive	14	12
negative	7	342

Sensitivity = 66%, specificity = 97%, predictive value of positive test = 53%, predictive value of negative test = 98%.

THE GENETICS OF ENDOTOXIN RESPONSIVENESS

Valuable insights concerning the mechanisms of endotoxin action have come from studies on endotoxin-hyporesponsive mice. The C3H/HeJ strain has a defect, localized to a single gene on the 4th chromosome, that confers resistance to essentially all of the manifestations of endotoxins (106). The nature of the abnormality has not been elucidated, but it is known that the defect can be repaired by replenishing lethally X-irradiated C3H/HeJ mice with bone marrow cells from normal mice (107). Not only are the responses of isolated macrophages and B-cells restored to normal, but the chimeric mice also become almost as sensitive (LD_{50} only 3-fold increased) as normal mice to the lethal toxicity of endotoxins. Thus it seems that virtually all of the responses to endotoxins involve one or more factors that can be replaced by bone marrow cells and that presumably require a single gene product. It is not clear at this time whether it is the marrow cells themselves that are critical for restoring sensitivity to high dose, lethal toxicity; possibly the bone marrow cells produce some humoral factor that is also missing in the C3H/HeJ mice. Serum-mediated phenomena have not been extensively studied in these mice, although there is a report that C3H/HeJ serum poorly opsonizes particles for phagocytosis (108). Recent studies strongly suggest that the defect in C3H/HeJ B-cells is localized to the plasma membrane (102), yet there is much evidence that the uptake of LPS by lymphocytes and macrophages of the hyporesponsive mice is normal. Interesting preliminary results by Ulevitch indicate that the missing factor may be a serine protease (or possibly a substrate that is altered so that it is no longer acted upon by the normal protease). If this hypothesis is correct, conceivably the critical endotoxin-activated molecule might be present in both cell membranes and blood.

It should be noted that C3H/HeJ cells are not totally incapable of responding to LPS. Indeed, macrophages from LPS-hyporesponsive mice migrate more rapidly in response to LPS than do macrophages from normal mice (109). These results support a two-signal model for LPS-cell interaction; most but not all of the responses to LPS require both signals.

LIPID A: STRUCTURE-FUNCTION RELATIONSHIPS

Several groups have recently determined the structure of lipid A from Salmonella, E. coli, Proteus, and other gram-negative bacteria. The Salmonella structure, shown in Figure 12, is representative of enterics.

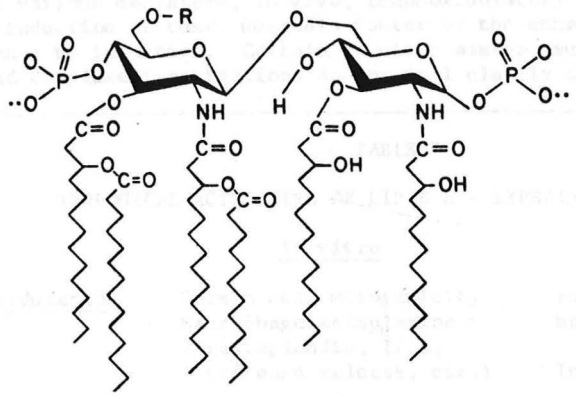


Figure 12. Lipid A structure, after ref. 110. R = H (R is the attachment site of the polysaccharide to lipid A in LPS)

Lipid A is a glucosamine disaccharide that has covalently linked phosphates and fatty acids. Phosphates are found at positions 1 and 4'; in some molecules these phosphates are substituted with additional groups such as phosphorylethanolamine, aminoarabinose, or additional phosphates. Four molecules of R-(+)-3-hydroxytetradecanoate are linked to the glucosamine backbone at positions 2, 3, 2', and 3'. The hydroxyl groups of some of these residues are further substituted with normal (nonhydroxylated) fatty acids; the acyl groups linked to glucosamine thus may have an acyloxyacyl structure (111).

Two recent developments have greatly advanced the understanding of the structure-function relationships of lipid A. First, biosynthetic precursors of lipid A have been isolated and the structures and biological activities of these molecules have been examined. Secondly, the organic chemical synthesis of lipid A has recently been achieved. Synthetic lipid A (Kyoto compound 506) and natural lipid A have identical biological activities (112,121). In contrast, the full range of biological activities is not produced by lipid A analogs that lack one or more critical groups. A consideration of the structure-activity relationships for both the biosynthetic precursors and the chemically synthesized analogs provides several clues to the molecular basis of endotoxin action.

The results of these studies support the concept, appreciated for many years by workers in the field (114), that endotoxins have both toxic and beneficial (immunomodulatory) activities. Toxicity is generally measured in

vivo by the ability of a preparation to produce fever in rabbits, to kill mice that have been adrenalectomized or sensitized with galactosamine, to kill chick embryos, or to elicit the dermal or generalized Shwartzman phenomena. Immunomodulatory activity may be measured in vitro by the ability of a preparation to stimulate spleen cells to proliferate or macrophages to release various mediators; in vivo, immunomodulatory activity can be measured by the induction of tumor necrosis factor or the enhancement of nonspecific resistance to infection. Certain in vitro assays, such as the Limulus lysate test and complement activation, do not fall clearly into either category.

TABLE 7

BIOLOGICAL ACTIVITIES OF LIPID A - EXPERIMENTAL TESTS

	In vitro	In vivo
Immunomodulation	Spleen cell mitogenicity Macrophage stimulation (Prostaglandin, IL-1, protease release, etc.)	Adjuvant activity Non-specific resistance to infection Induction of tumor necrosis factor
Toxicity		Pyrogenicity (rabbits) Dermal and generalized Shwartzman reactions Lethality (mice, chicks) Pulmonary hypertension, vascular leak (sheep)
Other	Limulus lysate test Complement activation (consumption)	

A cohesive picture of the structural requirements for various activities is limited because (1) some of the available compounds have not been tested in all of the assays, and (2) there has been some variability in the results of the assays (even those reported by the same group (115,116)). It is also likely that some differences in activity reflect, at least in part, differences in solubility and not structure. Nevertheless, the following observations seem fairly well substantiated at the present time:

(1) The precursor molecule known as lipid X (2,3 diacyl glucosamine-1-phosphate) is able to stimulate B-cells to divide and macrophages to secrete PGE_2 (117,118). Lipid Y, which is identical to lipid X with the exception that a molecule of hexadecanoate (palmitate) is substituted to the hydroxyl group of the 3-hydroxyltetradecanoate at the 2 position, also has immunomodulatory activity. Removal of the 3-hydroxytetradecanoate at the 3 position abolishes bioactivity in these assays.

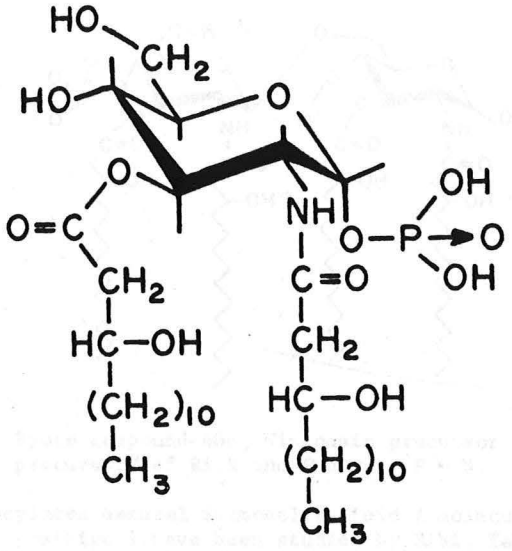


Figure 13. Lipid X
(Copyright. 1983. American Society of Biological Chemists, Inc. Takayama K, et al. J Biol Chem 258:14245-14252).

(2) The disaccharide precursor (the lipid A precursor, compound IVa, or precursor 1a) is a beta 1-6 linked glucosamine disaccharide that is phosphorylated at 1 and 4 and that has 3-hydroxytetradecanoate residues at positions 2, 3, 2', and 3'. The synthetic Kyoto compound 406 has the same structure (119,120). These compounds produce most of the immunomodulatory activities of lipid A. They stimulate B-cells and macrophages in vitro and they act as adjuvants in vivo (113,115,116,119). On the other hand, the compounds are unable to produce the dermal Shwartzman reaction and have reduced pyrogenicity when compared with complete lipid A.

The absence of a phosphate at position 1 or 4 (as in Kyoto compounds 404 and 405) does not greatly diminish the activity of the molecules in assays such as spleen cell (B-cell) mitogenicity or macrophage stimulation. It does make the compounds more anticomplementary, and it greatly reduces their activity in the mouse lethality test. Lack of <u>both</u> phosphates abolishes activity in all of the assays studied (Kyoto compound 403).

(3) By adding normal fatty acids to the hydroxyl groups of the 3-hydroxytetradecanoate residues at 3' and 2', the lipid A precursor (compound 406) is converted to complete lipid A (compound 506). The presence of acyloxyacyl groups greatly enhances the toxicity of the molecules, as measured by pyrogenicity and the dermal Shwartzman reaction (112,113). In fact, the presence of a single acyloxyacyl group at position 2 (as in the minor precursor 1b (122)) is sufficient to confer reactivity in the dermal Shwartzman reaction (123).

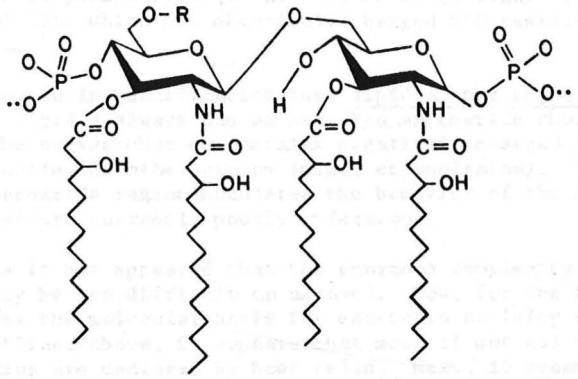


Figure 14. Kyoto compound 406, Wisconsin precursor IVa, the "lipid A precursor" of Rick and Osborn. R = H.

Fully acylated natural Salmonella lipid A molecules that lack the phosphate at position 1 have been studied by Ribi, Takayama, and others. These "monophosphoryl lipid A" molecules have greatly reduced toxicity (including minimal pyrogenicity in man (124)) yet they are able to stimulate spleen cells in vitro (117) and to induce tumor necrosis factor in vivo (125). Interestingly, unlike LPS or diphosphoryl lipid A, monophosphoryl lipid A does not suppress macrophage scavenger receptor activity for malondialdehyde-modified LDL (presumably a non-toxic bioactivity) (126). The synthetic compound that resembles "monophosphoryl lipid A" by lacking the 1-phosphate, Kyoto compound 504, seems generally similar in its properties, although it has been subjected to different tests (112).

The minimal requirements for lipid A activity in vitro thus appear to be met by a relatively simple diacylated monosaccharide. As Raetz has pointed out, lipid X has structural similarity to phosphatidic acid (127). Although the implications of this analogy to phospholipids are not entirely clear, early studies from his laboratory indicate that lipid X, like lipid A, can stimulate partially purified protein kinase C from macrophages (128). More recent studies indicate that the initial effect of LPS on intact pre-B lymphocytes is to stimulate phosphatidylinositol metabolism, casting doubt on a primary role for protein kinase C stimulation in these cells (168).

The studies described above suggest that the requirements for toxicity in vivo include (1) the presence of at least one acyloxyacyl group and (2) phosphates at 1 and 4'. Full acylation would be expected to enhance the hydrophobicity of the molecules and to increase the tendency of the molecules to aggregate with one another; aggregate size and/or shape might also be affected. The additional requirement for phosphates suggests that the expression of toxicity requires a negatively charged molecular "surface" that is only produced when the molecules are both fully phosphorylated and completely acylated. Presumably this charged surface can activate complement and the coagulation cascade (via Hageman factor or tissue procoagulants), thus initiating a complex series of interactions that lead to toxicity. If the critical requirement is for negative charge, and not for phosphate per

se, then the degree of phosphorylation may not be so critical for the toxicity of natural LPS, which has negatively charged KDO residues covalently attached to lipid A.

It should be noted in qualification that lipid A, per se, does not exist in nature; natural lipid A always has an attached saccharide chain. As mentioned above, the polysaccharide contains negatively charged sugars and has attached phosphates and other groups (e.g., ethanolamine). This hydrophilic polysaccharide region modulates the behavior of the hydrophobic lipid A in ways that are currently poorly understood.

For many years it has appeared that the enormous complexity of endotoxin effects would simply be too difficult to unravel. Now, for the first time, it seems likely that the molecular basis for endotoxin activity will be soon discovered. As outlined above, it appears that most if not all of the effects of endotoxins are mediated by host cells. Next, it seems likely that the product of at least one cellular gene is critical for endotoxin to stimulate cells; identification of this gene product is the subject of intense investigation in several laboratories. Finally, the minimal structural requirements for lipid A activity are essentially known, so that relatively simple probes may now be used to explore mechanisms.

There may also be practical benefits from this information. Many of the lipid A analogs lack toxicity yet are good adjuvants. It may be possible to use them to boost immune responses, or possibly to induce the release of various endogenous (i.e., made by host cells) mediators--such as tumor necrosis factor.

Two apparent contradictions deserve mention. First, endotoxin action appears to involve a critical gene; simple analogs of lipid A are able to stimulate cells, presumably by interacting with the product of this gene. Why should fully acylated lipid A have additional activity--i.e., toxicity? One explanation might be that the critical gene is necessary but not sufficient for the toxic activities. In other words, the gene product may provide the first step in the pathway to cellular stimulation, yet only the fully acylated lipid A can activate a second pathway that requires the first for its expression. Other evidence for a two-signal model is discussed above. Secondly, if the responses of animals to LPS are dose-related, why are there specific structural requirements for toxicity? One possible explanation is that the assays for lipid A bioactivity and toxicity are misleading. For most in vitro tests, the analogs are incubated with cells in the presence of small amounts of serum. For in vivo tests of pyrogenicity, lethality, and Shwartzman reactivity, in contrast, the analogs are injected intravenously or intradermally into animals. Since the different analogs vary considerably in their solubility, they may also differ in their tendency to disaggregate in vivo. Disaggregated LPS may bind to HDL and thus be less toxic than aggregated LPS (see the next section). A second possibility relates to the existence of enzymes in animal cells that remove the non-hydroxylated fatty acids from lipid A, essentially converting the molecule to the structure of a non-toxic analog (Kyoto compound 406) (see next section). When administered in low doses, the lipid A region of the LPS may be converted by the host into a non-toxic yet immunostimulatory form. Only with high doses would this detoxification mechanism be overwhelmed. These hypotheses are clearly speculative at this time.

DETOXIFICATION OF ENDOTOXINS

There appear to be both humoral and cellular mechanisms for LPS detoxification. The humoral mechanism involves the binding of LPS to HDL, since LPS that are bound to HDL have reduced bioactivity. Compared with the same number of unbound LPS, the LPS in LPS-HDL complexes are much less pyrogenic, do not induce leukocytosis or thrombocytopenia, and do not kill adrenalectomized mice (89,90). When given in high doses, on the other hand, these complexed LPS can produce hypotension and coagulation abnormalities in rabbits (78). The extent to which the LPS in the complexes become dissociated from the HDL over time is not known, although preliminary studies indicate that some dissociation does occur.

Presumably the HDL-bound LPS are less active because their lipid A moieties are sequestered by being inserted into the lipoprotein micelle. It is also likely, since LPS-HDL complexes do not stick to blood cells (78) and are not taken up by macrophages (99), that the LPS are effectively kept away from the usual target cells that initiate the host response.

Less is known about the cellular mechanisms for detoxifying LPS. Early studies indicated that mouse macrophages were able to detoxify LPS while neutrophils were not (129). More recent work has described a human granulocyte enzyme that specifically removes the non-hydroxylated fatty acids from lipid A (130). A similar enzymatic activity has been found in murine macrophages (131). Although this partial deacylation should detoxify the lipid A while preserving immunomodulatory activities, definitive tests of this hypothesis await purification of the enzyme. Macrophages appear to have other enzymes that remove 3-hydroxymyristate from the glucosamine backbone of lipid A, in keeping with the idea that they should completely inactivate the LPS.

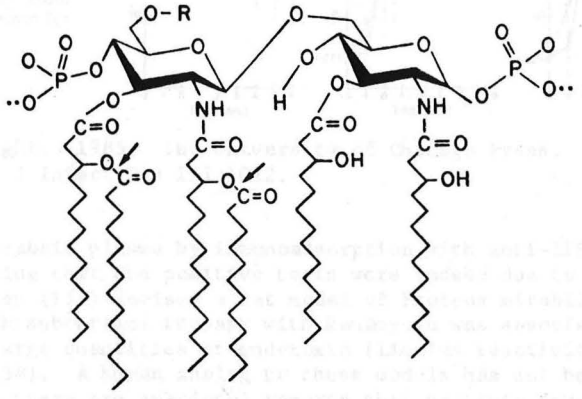
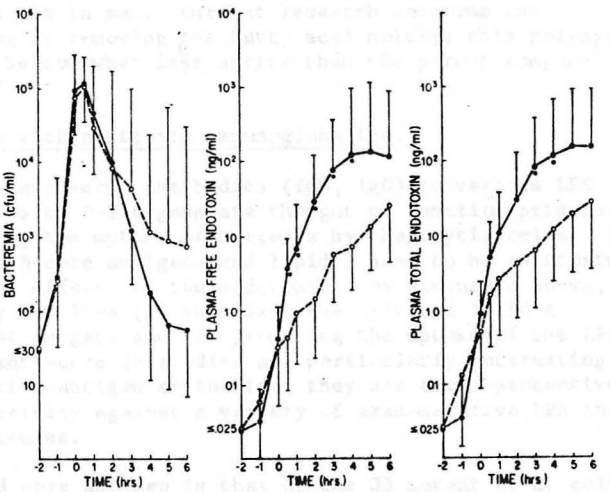


Figure 15. Site of action of acyloxyacyl hydrolase (arrow).

APPROACHES TO THE THERAPY OF ENDOTOXEMIA

1. Remove the source--eradicate the bacteria.

Although it would seem self-evident that killing the offending bacteria should be beneficial to patients with bacterial diseases, there is an interesting paradox that antibiotic therapy may actually increase the levels of circulating endotoxin. In rabbits with experimental E. coli bacteremia, administration of gentamicin or moxalactam decreased the number of viable bacteria in the blood while increasing the concentration of endotoxin that is measured by the Limulus test (132,133). It is also noteworthy that the treated rabbits recovered in spite of the endotoxin load--no adverse affects were attributed to the release of the endotoxin. The physical state of the endotoxin released by the bacteria was not studied--perhaps the LPS were rapidly bound to circulating HDL, so that the bioactivity of the molecules was reduced. It is also possible that the results reflect some artifact of the Limulus test system; the authors claimed that the Limulus reactivity was



Geometric mean levels of bacteremia (*left*), free endotoxin in the plasma (*center*), and total endotoxin in the plasma (*right*) in paired rabbits receiving either moxalactam (●) or gentamicin (○) at time 0. Animals were challenged ip with *E. coli* 2 hr before administration of antibiotic. Bars = 1 SD of the mean of six paired trials. * = statistically significant differences between the two treatment groups ($P < .05$, by the comparison of two means *t* test). The mean level of bound endotoxin for each treatment group is the difference in the mean levels of total and free endotoxin for the group.

Figure 16. (Copyright. 1985. The University of Chicago Press. Shenep JL, et al. J Infect Dis 151:1012.

removable from the rabbit plasma by immunoadsorption with anti-LPS antibodies, suggesting that the positive tests were indeed due to endotoxin. Johnston and Greisman (157) devised a rat model of Proteus mirabilis peritonitis in which suboptimal therapy with kanamycin was associated with the appearance of large quantities of endotoxin (Limulus reactivity) in plasma and death (134). A human analog to these models has not been described, although there are anecdotal reports that patients developed septic shock after the initiation of appropriate antibiotic therapy, and in 1958 Abernathy and Spink demonstrated that patients with brucellosis were hyperresponsive to injections of Brucella endotoxin (135).

Another approach to reducing the endotoxin burden involves sterilization of the gut. There is an experimental literature that suggests that neomycin bowel sterilization reduces endotoxemia in rats that are challenged with liver toxins (136), and clinical efforts to reduce endotoxemia by decreasing the numbers of bowel bacteria have also been undertaken (137). It is not possible to evaluate these studies carefully, but no striking beneficial effect has been reported.

2. Neutralization of LPS - the antibiotic polymyxin B.

Polymyxin B, a nonapeptide cationic antibiotic that has a lipophilic region, effectively neutralizes the bioactivities of LPS in many in vitro situations. It is thought to bind to the lipid A region of the LPS (138), possibly by hydrophobic interactions as well as the obvious charge-charge binding. The antibiotic is also bactericidal toward many gram-negative bacteria. Attempts to evaluate the ability of polymyxin B to neutralize LPS in vivo have met with mixed results (139,140,141). In any case, the toxicity of the drug limited its use in man. Current research concerns the modification of the drug by removing the fatty acyl moiety; this polymyxin B nonapeptide appears to be somewhat less active than the parent compound in most respects (142).

3. Neutralization with antitoxin immunoglobulins.

Most human beings have serum antibodies (IgM, IgG) to various LPS antigens. The antibodies to O-antigens are thought to function principally as opsonins, facilitating the uptake of bacteria by phagocytic cells. In contrast, antibodies to R-core antigens and lipid A seem to be antitoxins, neutralizing the various effects of the endotoxin. As discussed above, this neutralization probably involves (1) shielding the critical lipid A structures from key host targets and (2) promoting the uptake of the LPS by phagocytic cells. The anti-core antibodies are particularly interesting because, unlike the anti-O-antigen antibodies, they are cross-protective, expressing antitoxic activity against a variety of gram-negative LPS that have similar core structures.

One widely studied core antigen is that of the J5 mutant of *E. coli* O111. This antigen has the following general structure:

$$\text{glucose} - (\text{heptose})_2 - (\text{2-keto-3-deoxyoctonate})_3 - \text{lipid A}$$

Another important core antigen is that of the Re mutant of *Salmonella minnesota*; this antigen has only the KDO residues attached to lipid A.

Recent studies indicated that most individuals acquire serum IgG and IgM antibodies to the J5 glycolipid by the age of two years (143), presumably from auto-immunization by bacteria in the gut. Moreover, retrospective clinical studies have shown that high titers of serum antibodies to Re or J5 antigens at the onset of sepsis are associated with a greater likelihood of surviving the septic episode (144,145).

Clinical application of these observations has now been achieved, largely through the efforts of Abraham I Braude and his coworkers (reviewed

in 146). Braude envisioned that an antiserum to the J5 antigen would produce cross-protection against the variety of gram-negative bacteria that produce clinical sepsis. Early studies established that antisera to these mutants could protect animals that were either injected with lethal doses of LPS or infected with gram-negative bacteria. The protection was largely independent of the challenge strain; J5 antiserum could protect animals from organisms such as P. aeruginosa, E. coli, and meningococci. Human antiserum, produced by immunizing San Diego firemen with boiled J5 cells, was shown to be an effective antitoxin in animals.

The landmark clinical study was performed by E. Ziegler, A. Braude, and many collaborators and reported in 1983 (147). In a randomized, double-blind clinical trial they administered either human J5 antiserum or preimmune serum to 191 severely ill patients with presumed gram-negative bacterial sepsis. The results indicated that the antiserum significantly improved the outcome of patients who were bacteremic (24% mortality vs. 38% in controls), particularly if there was profound shock (46% mortality vs. 76% in the control group). Interestingly, patients who had established gram-negative bacterial infections but negative blood cultures seemed to be benefited by the antiserum (44% mortality in the untreated group vs. 8% mortality in the antiserum group--the numbers of patients were small and the difference was significant at $p = 0.08$).

Subsequent clinical studies. Recent evidence indicates that human J5 antiserum, given prophylactically, can reduce the severity of gram-negative bacterial infections in surgical patients with severe trauma or abdominal surgery (148). Interestingly, the frequency of local gram-negative infections was not different in the test (J5 antiserum) and control (preimmune plasma) groups. Rather, there was an impressive reduction in the frequency of shock and death from gram-negative sepsis in the antiserum recipients. The protective effect was particularly striking in patients who had had abdominal surgery.

TABLE 8

TRIAL OF PROPHYLACTIC/THERAPEUTIC J5 ANTISERUM IN SURGICAL PATIENTS (140)

	J5	Control	Relative risk	p
Abdominal surgery n =	71	83		
Gram-negative infection	8	15	1.6	NS
Shock	2	13	5.6	0.006
Lethal shock	1	9	7.7	0.017
All patients n =	126	136		
Gram-negative infection	16	23	1.3	NS
Shock	6	15	2.3	0.049
Lethal shock	2	9	4.2	0.033

In this study the J5 antiserum was given on admission to the surgical intensive care unit and again every 5 days and at the onset of septic shock. Since patients with abdominal surgery and septic shock did not benefit from J5 antiserum when it was given only therapeutically (147), the results of the combined prophylaxis/therapy trial support the notion that lethal shock develops in patients who are depleted of protective antibody.

In contrast, J5 antiserum apparently provided no significant protection when it was used prophylactically in neutropenic patients; the details of this study have not been published.

A somewhat different approach has been taken by other workers. Lachman et al (South Africa) (149) and Marget et al (Munich, West Germany) (150) screened units of plasma for antibodies to the LPS O antigens or lipid A, respectively, and pooled units that had high antibody activity. When administered to septic patients, the antiserum appeared to have protective efficacy. Neither study was properly controlled (151). Elisa methods for screening plasma units for anti-J5 antibodies are now widely available, and it seems likely that further attempts will be made to produce high-titer pooled antiserum for clinical use.

Controversy: the mechanism of the protective effect. The San Diego workers argue that the J5 serum contains antibodies to the J5 antigen that function primarily as antitoxins; a similar view is held by the Boston group (152). Other workers have held that the protective effect of J5 antiserum might be produced by some component of the serum other than immunoglobulin, or by antibodies that were recalled in the vaccine recipients by immunization with the whole J5 bacteria (anamnestic responses) (153). Two observations provide some clarification of this issue. First, whatever the nature of the protecting substance, it can apparently be adsorbed from the serum with J5 LPS. Second, a human IgM monoclonal antibody raised to J5 LPS (which apparently has binding specificity for lipid A) also provides cross-protection toward LPS and lethal bacteremia in experimental animals (154). (In contrast, other workers have produced mouse IgG monoclonals to J5 that do not have cross-reactivity with smooth LPS (155); the explanation for this discrepancy, while unclear at the present time, may relate to the immunoglobulin class, epitope specificity, or species of origin of the monoclonals. Some workers also privately express doubt about the reproducibility of the reported findings using the human IgM monoclonal.)

The San Diego and Boston workers contend that the anti-core or anti-lipid A antibodies mask the lipid A region and prevent it from interacting with the host mediators of shock. A role as an opsonin, promoting clearance of bacteria from the blood, seems less important. Evidence for this includes (1) the inability of J5 antiserum to enhance killing of gram-negative organisms or to promote clearance of the organisms from the blood, and (2) the clinical observation that patients given prophylactic J5 antiserum appear to be protected from shock but not from local infections. A recent study found that rabbit J5 antiserum promoted bacterial clearance but provided no protection from injected LPS (153); an explanation for this difference is not readily apparent.

Prospect. Clinical trials of the human IgM monoclonal antibody should be underway shortly. Other approaches to providing a standardized

preparation of J5 antiserum include the pooling of high titer plasma units and the preparation of lyophilized human antiserum. It should be noted that human gamma globulin preparations are IgG immunoglobulins; preparations of IgM from human plasma are not available using existing technology. If the current hopes are realized, some form of J5 antiserum should be available for general clinical use within a few years.

4. Prevent the synthesis or release of mediators by cells

a. High dose glucocorticoid therapy.

The history of the use of glucocorticoids as adjunctive therapy for gram-negative bacterial sepsis has been discussed in several recent reviews (155,162) and will not be repeated here. The rationale for steroid therapy is based on considerable evidence in animals and man that these drugs may interfere with the inflammatory response to gram-negative bacteria. Although glucocorticoids are known to block many of the effects of LPS on cells, the details of their many antiinflammatory effects have not been elucidated.

Large doses of glucocorticoids will prevent endotoxic shock in animals. The drugs are most effective when they are given before the endotoxin challenge, but some benefit has been reported when steroids have been given as long as several hours after a bolus of LPS. Moreover, in two animal models of gram-negative bacteremia there is evidence that steroids prevent death when used in combination with an aminoglycoside. The most informative animal model is that of Greisman (134,157). Mice are inoculated intraperitoneally with a carefully titrated dose of gram-negative bacteria, followed in a few hours by a dose of kanamycin that will protect approximately 50% of the animals. When methylprednisolone therapy is added to the antibiotic, significant protective benefit can be shown. Greisman and his colleagues have shown further that the antibiotic therapy produces an increase in the level of circulating endotoxin (limulus lysate activity) in the animals, and they propose that the beneficial effect of the steroid is to prevent the toxic responses to this antibiotic-released endotoxin (158). In a different model, Hinshaw infused an LD_{100} dose of live E. coli into baboons. Treatment after 3 hours of hypotension with gentamicin and methylprednisolone significantly improved survival in this model. It was not clear whether the antibiotic or the steroid was more important in producing this effect, however.

In 1976, Schumer reported the results of a study that evaluated the efficacy of high-dose glucocorticoid therapy in human septic shock (159). A highly beneficial effect of steroids was described. Various problems with his methods were noted by others (155), however. In response to much demand for a better study, the Veterans Administration initiated a collaborative trial of high-dose (30 mg/kg) methylprednisolone therapy in human patients with septic shock. The goal of this study is to determine the efficacy of methylprednisolone therapy in specific patient subgroups, with particular emphasis on the time of administration of the steroid (with respect to the onset of shock) and underlying host parameters that might permit some selectivity in the use of the drug. A beneficial effect was strongly predicted publicly by the organizer of the trial in 1983, before the study was begun.

A smaller study was published from the Miami, Florida VA in 1984 (160). Fifty-nine patients who met the admission criteria for septic shock were randomly assigned to receive methylprednisolone (30 mg/kg), dexamethasone (6 mg/kg), or nothing (no placebo preparation was described). The steroid was administered at the time of entry into the study and, if no improvement was noted, again 4 hours later. Patients in both control and drug groups received pressors, antibiotics, and fluids. Although the state of shock was reversed more frequently in the steroid groups than in the control group, no significant difference in overall mortality was found. If the 11 patients who died within 12 hours after drug therapy were excluded, reversal of shock occurred in 71% of the steroid-treated patients with rapidly fatal or ultimately fatal underlying disorders and in 14% of the controls in these disease categories. Again, no difference in ultimate outcome was found. Shock reversal was more common if the drug was begun within 4 hours of the onset of shock (73% in steroid groups vs. 20% in the controls), whereas there was no difference in the frequency of shock reversal in steroid and control patients who were treated more than four hours after the onset of shock. Again, early administration of steroids had no apparent effect on mortality. Superinfections were more common in the steroid-treated patients but the infections noted were not usually life-threatening (161).

This randomized trial thus did not strongly support the use of steroids in septic shock (162). It did suggest that steroids, given early in the course of shock, might provide temporary benefit to some patients so as to allow other measures (such as surgical drainage or antibiotic therapy) to have an effect. Use of steroids in patients who have been in shock for longer than 4 hours cannot be supported by the available data.

The results of the VA collaborative trial are awaited, and it is hoped that the larger numbers of patients will allow further definition of the role of steroids in treating septic shock.

b. Non-steroidal antiinflammatory agents (indomethacin, aspirin)

Although there is much information about the beneficial effects of these agents in experimental endotoxic shock in animals (see page 10), no trials in man have been reported and, perhaps most importantly, even in the animal models these drugs must be given before the endotoxin for a beneficial effect to be observed.

5. Counteract the consequences of mediator release

a. Anticoagulation

As discussed on page 19, heparin therapy is rarely indicated in patients with sepsis-related DIC. Most authorities prefer to administer fresh frozen plasma to patients who are bleeding.

b. Naloxone

The recently reported human trial of naloxone in patients with septic shock is described above (page 14). Preliminary word from the organizers of a subsequent trial (using 10 mg naloxone) suggests that the higher dose is also not efficacious.

c. Fluids, pressors. These mainstays of therapy have not been evaluated by controlled trials in patients with septic shock.

Summary. It should be emphasized again that septic shock is an extremely complex derangement. The responses to endotoxin and other bacterial products are greatly modified by the underlying condition of the host. We should not expect single interventions to produce dramatic results; small but clinically significant differences between treated and control groups may require larger study populations than have been used. Moreover, the clinical utility of various interventions may be confined to rather special subgroups, and these subgroups need to be identified in prospective, randomized studies. This field has had a slow beginning and much remains to be done.

It should also be obvious that interventions (such as antiserum or glucocorticoids) that prevent the release of mediators are likely to be more important than measures (such as naloxone) that reverse the effects of only one of the mediators.

SUMMARY: TOXIN OR TONIC?

Gram-negative bacterial lipopolysaccharides are ubiquitous molecules that may stimulate both beneficial and toxic responses in animals. It appears that the response to LPS is largely a matter of degree: toxicity is due to many of the same mediators that, when released in smaller amounts, are usually beneficial to the host.

It may be possible to carry this argument even further and suggest that, in very low doses, LPS prime certain components of the immune response in normal animals. In other words, LPS from the gut, when absorbed in small amounts into the portal circulation, may modulate the "set-point" for various immune responses. Evidence for this hypothesis comes from several sources:

(1) Endotoxin-hyporesponsive mice have exaggerated immunoglobulin responses to antigens that are administered via the gut. Work by McGhee and his colleagues has shown that these mice have puny suppressor T cell responses; the defect maps to the LPS^d gene (163). These workers hypothesized that in normal animals, LPS induces T-suppressor cells in Peyer's patches that suppress polyclonal B cell responses to orally administered antigens.

(2) Germ-free mice are quite resistant to LPS toxicity, although in vitro their B-cells are hyperresponsive to LPS (164). (Germ-free animals are not strictly endotoxin-free, since LPS are present in sterilized food.)

(3) Neutrophils and macrophages require low levels (picograms/ml) of LPS to achieve "normal" responses to various stimuli in vitro (165,166).

It is thus possible that, depending on the dose and the manner of presentation, bacterial lipopolysaccharide may be either tonic or toxic to animals.

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