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J C Hurley
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Endotoxemia: Methods of Detection and Clinical Correlates

JAMES C. HURLEY*

Division of Infectious Diseases, Children's Hospital & Medical Center, Seattle, Washington

* Mailing address: Clinical Microbiology and Infectious Diseases, Alfred Hospital, Commercial Rd., Prahran, Victoria, 3181, Australia.

INTRODUCTION

> Endotoxins possess an intrinsic fascination that is nothing less than fabulous. They seem to have been endowed by Nature with virtues and vices in the exact and glamorous proportions needed to render them irresistible to any investigator who comes to know them. . . . Not only do endotoxins elicit striking effects by themselves, but they possess almost limitless ability to potentiate or antagonize or to be potentiated or antagonized by the action of other agents or states including adrenal steroid hormones, catecholamines, ionizing radiation, pregnancy, hemorrhage, dietary manipulation, high environmental temperatures, and so called reticuloendothelial cell blockade. . . . Even when we have accomplished the difficult task of defining the mode of action of endotoxins in man, there will remain for many of us an additional problem. This will be to determine the actual significance of endotoxins in human health and disease, not in the controlled environment of the research laboratory but in daily life, in the field, in nature as we have modified it. Endotoxins can cause fever, but how many human fevers are endotoxic? Endotoxin can cause shock but how often is shock in man endotoxic?
>
> Ivan L. Bennett, 1964 (22)

The similarities between the pathophysiology of gram-negative sepsis and the pathophysiology induced by the administration of endotoxin in the "controlled environment of the research laboratory" have been reviewed in detail elsewhere (33, 54, 118, 221, 229, 233, 248, 249, 422). The aim of this review is to survey the published experience "in the field," using the *Limulus* amebocyte lysate (LAL) assay for the detection of endotoxin in various clinical contexts with particular attention to the detection of endotoxemia. It should be noted that the LAL test has yet to gain Food and Drug Administration approval for the detection of endotoxemia in clinical practice, and currently licensed kits specify that they are not approved for that purpose.

Endotoxins: Structure-Activity Relationships

Endotoxins are large (molecular weight, 200,000 to 1,000,000), heat-stable (to 100°C) lipopolysaccharides (LPS) which are the major components of the cell wall of the gram-negative bacterium (Fig. 1). While the terms endotoxin and LPS are used interchangeably, the former term to emphasize the biological activity and the latter term to refer particularly to the chemical structure and composition of the molecule, it should be noted that not all bacterial LPS are endotoxins (146, 307) and not all "endotoxin" (especially that derived by trichloroacetic acid extraction, in which significant non-LPS cell wall components are incorporated) is LPS (229).

The structure is characteristic (316). It consists of lipid A (Fig. 2) (a diglucosamine unit that contains fatty acids which have a 10- to 20-carbon-atom chain length), a carbohydrate core, and the polysaccharide O antigen (repeating sequences of either linear or branched component sugars which vary in chain length among the various strains of bacteria). Studies using X-ray crystallography suggest that the lipid A component of the molecule is in a highly ordered conformation within the outer membrane of gram-negative bacteria and it is relatively concealed within this membrane, where it presumably has an important role in maintaining structural integrity (203) (Fig. 1).

Endotoxin induces multiple biological effects in vivo, for example, fever, leukocytosis, hypoferremia, platelet aggregation, thrombocytopenia, and coagulopathies (229, 233, 248). These effects can be attributed to activation of various endogenous pathways or cascade mechanisms. For example, LPS triggers the complement, coagulation, fibrinolytic, and kinin pathways to release vasoactive peptides and also the release of an array of cytokine mediators from macrophages and monocytes. The release of these mediators in turn triggers the characteristic biological effects. Nearly all of these effects are mediated through the lipid A region. It is this lipid A region of the molecule that is reactive with the LAL assay, the rabbit pyrogen test, and other bioassays. Immunoassays, on the other hand, recognize the biologically relatively inert polysaccharide region of the endotoxin molecule.

Despite this uniformity in the structure of the lipid A component of the LPS molecule from diverse gram-negative bacteria, its potency and spectrum of activity are not a uniform gravimetric property of LPS. The results of extensive studies with synthetic lipid A and partial structures of lipid A have indicated the minimal molecular requirements for endotoxin activities in the lipid A molecule (Fig. 3) (303, 316, 317). The

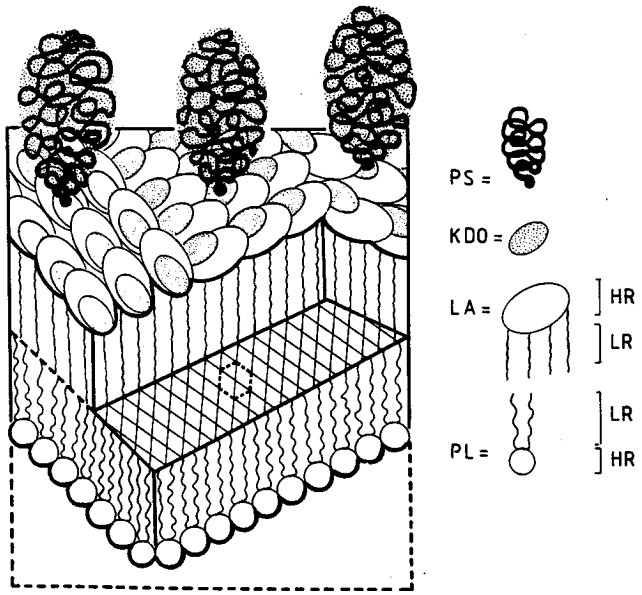

FIG. 1. Schematic view of the outer membrane of gram-negative bacteria to illustrate the relative placements of the various components in relation to the external environment (top). PL, phospholipids; LA, lipid A (HR, hydrophilic region; LR, lipophilic region); KDO, 3-deoxy-D-manno-2-octulosonate; PS, polysaccharide. The model depicted is intended to illustrate the following: the high state of order of LPS within the outer membrane compared with the phospholipid arrangements found in cytoplasmic membranes, the heavily coiled configuration of O-specific chains, and the shed-roof-like structure of the lipid A moiety. Reprinted from reference 203 with permission of the publisher.

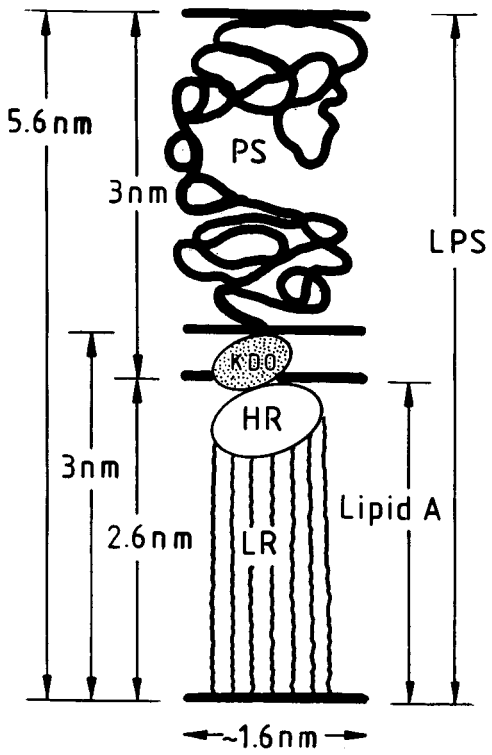

FIG. 2. Schematic view of a *Salmonella* sp. smooth-form LPS molecule. The dimensions of the individual regions were derived from X-ray diffraction data. Abbreviations are given in the legend to Fig. 1. Reprinted from reference 203 with permission of the publisher.

potency of extracted LPS is also influenced significantly by physical (200) and solubility (348) characteristics that determine its macromolecular aggregation.

The Concept of Free Endotoxin

The term endotoxin itself implies that within each cell there is a quanta of endotoxin whose activity is dependent on cell lysis. However, for several reasons, this concept of endotoxin is unhelpful. It should be noted that the term is a double misnomer as neither is it strictly endogenous nor is it a toxin as the term might imply (372, 373). The level of endotoxin as measured by the LAL assay closely parallels the density of bacteria throughout logarithmic growth, and shedding of cell-free endotoxin occurs spontaneously and without cell lysis (72, 150, 189).

The process by which gram-negative bacteria release endotoxin in the absence of lysis can be accentuated by various environmental factors (158, 330) and the action of antibiotics, including inhibitors of protein synthesis (4, 172, 326). The release of LPS following antibiotic action is not limited to antibiotics that directly affect the cell wall, such as beta-lactam antibiotics (160, 163a, 326). For example, inhibitors of protein synthesis, such as chloramphenicol, increase the release of LPS (4), and this probably occurs as a consequence of the effects of chloramphenicol on the mechanisms that control the production of LPS (172). Gram-negative bacteria with a rough LPS phenotype shed as much as 10 times more endotoxin than do gram-negative bacteria with a smooth LPS phenotype (189, 191, 224). The rate of shedding is in general higher for serum-resistant or virulent variants of *Neisseria gonorrhoeae* (70) and *Neisseria meningitidis* (5, 242, 341) than for avirulent isolates. Indeed, the meningococcus may shed as much as 18% of the total endotoxin by this process (72). A more helpful concept of endotoxin release therefore might be as an increase in its bioavailability, a process that should not be considered dependent on cell lysis.

The question of bioavailability of endotoxin in the setting of gram-negative sepsis has become complicated with the recent recognition of host-derived proteins such as lipoproteins (90), bactericidal permeability-increasing protein (BPI), LPS-binding protein, septin, and CD14 which function as endotoxin receptors in that they bind to LPS to modulate its activity and its clearance from the circulation (89, 223, 414). Levels of specific lipoproteins correlate with the ability to recover endotoxin from plasma with the LAL assay (90). The serum protein LPS-binding protein augments the production of cytokines by facilitating the delivery of LPS to the LPS-binding glycoprotein CD14 in the plasma membrane of cytokine-producing monocytes. In contrast, BPI, a cationic protein derived from azurophilic granules of polymorphonuclear neutrophils and N-terminal fragments derived from BPI bind specifically and with high affinity to LPS in the cell envelope of gram-negative bacteria both to effect bactericidal antibacterial activity and to inhibit the activity of LPS (284). The ratio between LPS-binding protein and BPI has been found to vary between serum and other noninfected fluids on the one hand and abscess cavities on the other (284a).

CLINICALLY APPLICABLE METHODS FOR ENDOTOXIN DETECTION

There are more than 20 assays for the detection of endotoxin (229), of which three have been used for the detection of endotoxin in clinical specimens: the rabbit pyrogen assay, the LAL bioassay, and immunoassays. The method of choice would appear to be the LAL assay. The advantages of this assay are increased sensitivity, potential for quantitation, reactivity with the biologically active component lipid A, and relative convenience of operation.

The LAL Assay

In 1956, Bang (15) discovered that the endotoxin of a *Vibrio* species from seawater, pathogenic for the horseshoe crab (*Limulus polyphemus*), caused fatal intravascular coagulation and that endotoxin induced activation of this process in vitro. Levin, Bang, and coworkers subsequently showed that this coagulation was the result of an endotoxin-initiated reaction causing the enzymatic conversion of a clottable protein derived from the circulating blood cell (amebocyte) of the crab (207, 423). They recognized the potential for this biological reagent as a diagnostic tool and characterized its properties. A lysate from the amebocyte is extremely sensitive to the presence of endotoxin; however, four aspects of this reagent present difficulties: (i) quantitation of LAL enzyme activity is complicated by the sigmoidal shape of the reaction curve; (ii) there is a requirement for optimal conditions, which is exacting; (iii) measurement of endotoxin in plasma is complicated by the presence of various unidentified interfering factors; and (iv) the level of endotoxin in blood is generally at the limit of sensitivity of the assay.

Over the subsequent two decades, several approaches to circumvent these difficulties have been described (reviewed in reference 273). An analogous assay derived from *Tachypleus tridentatus* amebocyte lysates has been described (386).

Coagulation system of *L. polyphemus*. The coagulation system of *L. polyphemus* consists of several enzymes which are

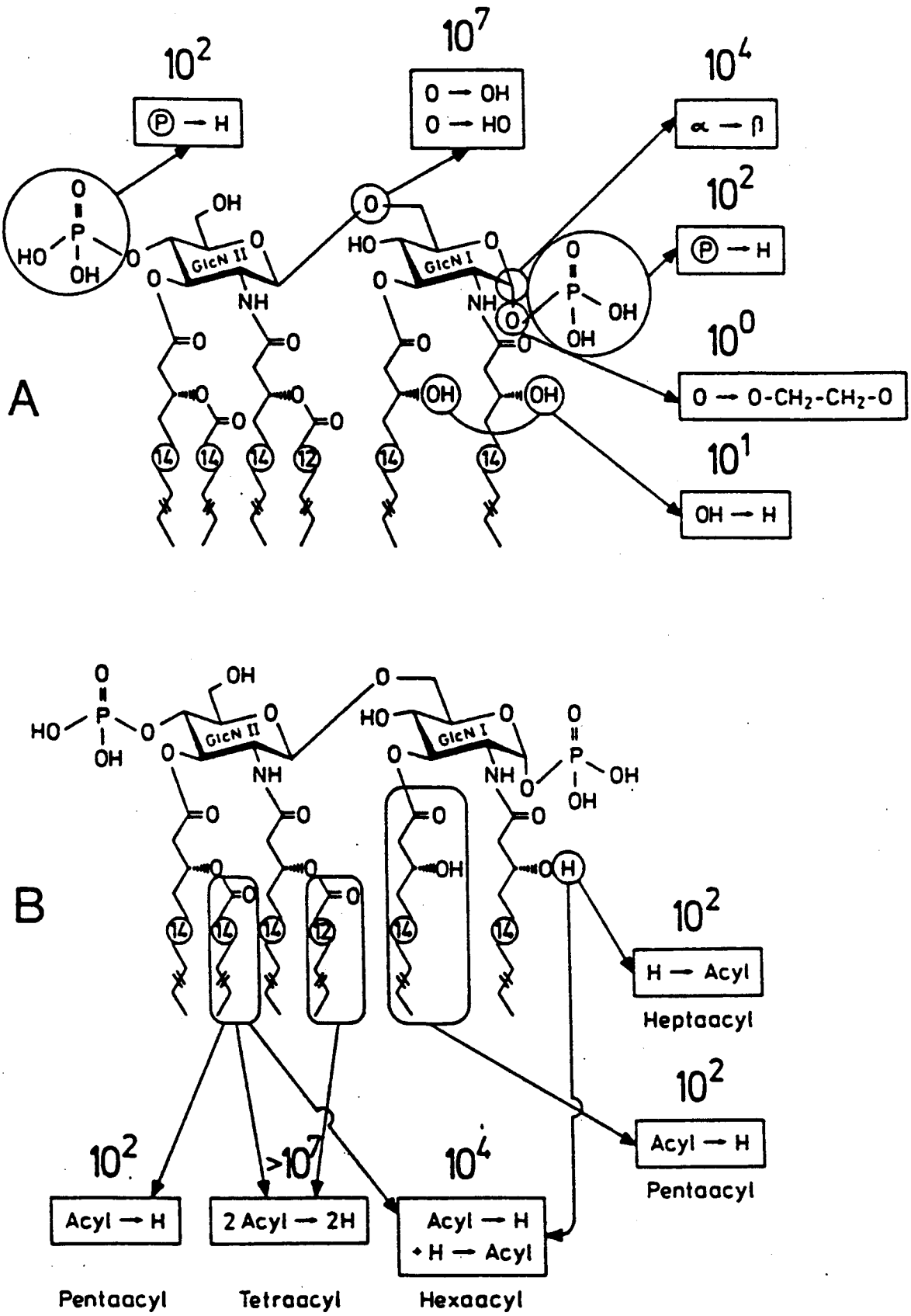

FIG. 3. Schematic representation of lipid A structure-activity relationships. Shown are chemical changes of the *E. coli* lipid A structure and the factor by which the structure generated is less active than lipid A as determined by their ability to activate the production of mononuclear cell peptide mediators. (A) Modifications of the hydrophilic region of lipid A. (B) Modifications of the hydrophobic region of lipid A. Reprinted from reference 316 with permission of the publisher.

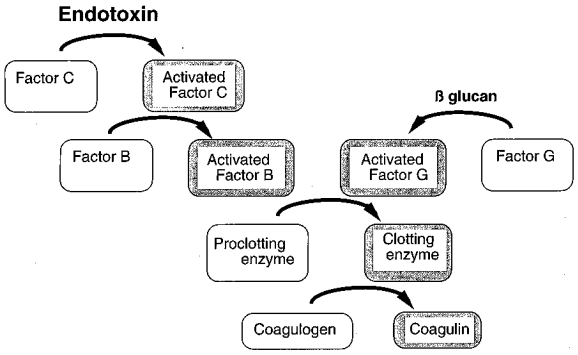

FIG. 4. Schematic representation of the LAL gelation reaction showing the endotoxin-activated pathway leading to coagulogen conversion together with an alternate (1-3)-β-D-glucan-activated pathway. Chromogenic substrate, when present, is susceptible to cleavage by the activated clotting enzyme.

arranged in three pathways in a fashion which resembles the classic, alternate, and common mammalian coagulation cascade pathways, the components of which activate each other in a "cascade" sequence. The coagulation system of the Japanese horseshoe crab, *T. tridentatus*, which is considered homologous to the *L. polyphemus* American horseshoe crab, has been studied extensively (Fig. 4) (173, 175). This cascade sequence results in an amplification of the original stimulus which accounts for the sensitivity of the *Limulus* coagulation system to endotoxin at picogram-per-milliliter (10^{-12} g/ml) concentrations. An additional component of *Limulus* amebocytes is an anti-LPS factor which has anti-endotoxin properties (395).

Gel clot LAL assay. In the original version of the gel clot test, the endotoxin-activated clotting enzyme cleaves the coagulogen to form a clot. To perform this test, a small amount of LAL solution is added to an equal volume of a sample or a standard dilution in a small test tube. If, after an appropriate incubation time, a firm gel clot is formed, the test is scored positive. A firm gel clot is one that remains solid in the bottom of the reaction tube when the tube is inverted. Methods to enhance the visualization of clot formation in microtiter volumes have been described (113, 167, 299). With all gel clot-based techniques, a semiquantitative result can be obtained through serial dilution of samples and standards.

Coagulogen-based LAL assay. The limitations of the gel clot LAL test are the subjective endpoint and the relative lack of sensitivity. To overcome these limitations, various methods to quantitate the progress of the reaction leading to coagulogen conversion have been employed, for example, through monitoring the increase in turbidity (79, 385), the loss of coagulogen as the clot forms (10, 425), the increase in precipitated protein (263, 264), or the appearance of a peptide cleavage fragment of coagulogen (426).

Chromogenic LAL assay. In the chromogenic LAL assay method (174), the coagulogen is completely or partially removed to be replaced by a chromogenic substrate (342), a small synthetic peptide linked to a chromophore (*para*-nitroaniline) containing an amino acid sequence similar to that present at the site in the clotting protein cleaved by the clotting enzyme (X-Y-Gly-Arg-pNA). The chromogenic LAL assay usually has two stages: a LAL activation stage and, following the addition of the chromogenic substrate to the reaction mixture, a chromophore release stage. Release of the chromophore imparts a yellow color to the solution. The strength of the yellow color (as measured by optical density [OD] at 405 nm in a spectrophotometer) is a function of the amount of active clotting enzyme (and indirectly to the amount of endotoxin) present in the solution. Both phases of the chromogenic reaction are critically time and temperature dependent, but within these limitations the chromogenic assay is sensitive to 10 pg/ml (375). A single-step chromogenic assay has been described (81, 215).

Specificity of the LAL Assay

There are conflicting reports regarding the specificity of the LAL assay with some reporting reactivity with cell wall products of fungi, gram-positive bacteria, and polynucleotides (88, 278, 378). The extreme sensitivity of the LAL assay is a factor that confounds the assessment of its specificity. For example, positive results in the LAL assay with extracts of *Candida albicans* were attributed to endotoxin contamination of the microbial growth media from which these candida cells had been harvested (71).

In general, only LPS can produce a positive LAL assay at concentrations as low as picograms per milliliter. When reactions with other microbial products were reported, for example, peptidoglycan derived from the cell walls of gram-positive organisms (195, 406) or (1-3)-β-D-glucans (322, 425), the concentrations required were 1,000 to 400,000 times higher than the required concentration of endotoxin. Accordingly, contamination of peptidoglycan with 0.00025% endotoxin could account for a positive LAL assay and is difficult to exclude. Others report no LAL reactivity for the same compounds (416).

The two pathways leading to the coagulation of LAL, one activated by endotoxin triggered by factor C and the other activated by β-glucans triggered by a glucan-reactive factor G, can be specifically blocked by polymyxin and laminarin, respectively (424). Hence, reactivity with the LAL assay that is inhibited by polymyxin B can be used as specific evidence for endotoxin.

The reactivity of the LAL assay with fungal wall material has been an inconsistent finding with LAL prepared from the American horseshoe crab (149, 322). By contrast, LAL reagent prepared from Japanese crabs is more consistently reactive with β-glucan, and this reactivity has been attributed to the factor G-triggered alternate pathway (149). LAL derived from the Japanese horseshoe crab and from which this factor G has been removed has been promoted as an endotoxin-specific reagent (278, 279). However, in an evaluation of a conventional LAL test and a factor G-free LAL test that included seven patients with documented candidemia, similar results were obtained in both assays (67).

The practical significance of this nonspecificity for LPS in clinical testing is unclear. For fluids other than blood, such as urine and cerebrospinal fluid (CSF), the assay is very specific. In four studies of urine testing compiled by Elin and Hosseini (85), only two positive LAL tests were found in 85 urine samples from patients infected with gram-positive bacteria. Similarly, in 19 studies of LAL tests on CSF specimens from patients with culture-documented meningitis compiled by Nachum (258), only 3 of 210 specimens from patients with meningitis due to gram-positive bacteria, mycobacteria, or a spirochete were LAL positive.

In the two literature surveys by Elin and coworkers (84, 85), there are 27 studies in which the results of the LAL test of plasma are given in relation to the finding of a gram-positive infection. While a positive LAL test was found among 18 of 149 patients, 16 of these positive tests came from only two studies (86, 358). Inevitably, with LAL assays sensitive to less than 1-ng/ml concentrations of LPS, samples with false-posi-

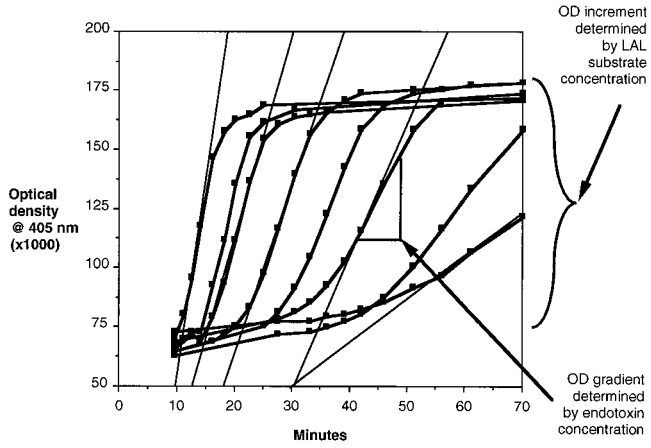

FIG. 5. LAL reaction curves showing the progress of the endotoxin-activated gelation of LAL at eight concentrations of endotoxin (from right to left, respectively: 0.033, 0.1, 0.33, 1, 3.3, 10, 33.3, and 100 ng/ml) as change in OD at 405 nm in a microtiter tray (by the method described in reference 166). The maximal gradient of OD increase is superimposed on alternate reaction curves. The overall shape of each curve is sigmoid, the final OD is a function of the concentration of LAL substrate (i.e., coagulogen or, when present, chromogenic substrate), and the rate of increase is a function of the concentration of endotoxin.

tive results due to contamination from exogenous LPS will be difficult to exclude.

Positive LAL reactions of plasma have been described in patients with malaria (211, 381), especially cerebral malaria (387). The basis for this association is unclear. On the one hand, LAL reactivity has been described with soluble antigens of *Plasmodium falciparum* (178), whereas on the other hand, the incidence of gram-negative bacteremia in the endotoxemic malaria patients of one study was surprisingly high (13 of 43 [387]). Hence, the unexpected finding of positive LAL tests in any setting may represent occult polymicrobial infections.

Optimal Conditions for LAL Reaction

The activities of the LAL enzymes are dependent on optimal reaction conditions of pH, ionic content, and temperature, and these have been empirically determined (55, 325, 342, 359). These conditions are additional to those that modify the activity of endotoxin itself. The composition of some biological fluids, in particular, urine, may cause false-negative tests if undiluted samples are tested (262). While changes in one LAL reaction parameter, such as time of incubation, can compensate for changes in another, such as pH, attention to consistency in reaction conditions will be an important consideration toward the interassay comparisons of quantitative results.

Endpoint and kinetic quantitation of LAL assay. Levin and Bang (207) described three properties of the progress of the LAL gelation reaction when monitored as a change in OD. These three properties need to be considered in the design of a quantitative assay for endotoxin (Fig. 5). (i) The progress of the reaction follows a sigmoid curve, with an initial plateau, a phase of rapid rise, and a terminal plateau. (ii) The absolute increase in OD is determined by the concentration of LAL clottable protein. (iii) The rate of increase in OD is determined by the concentration of endotoxin (423).

The progress of the LAL assay can be monitored in two ways, using endpoint or kinetic methodology. With endpoint methodology, the OD is recorded at only a single time point, usually 30 min. Because of the sigmoidal shape of the reaction curve, the relation between OD and endotoxin concentration at a given time point is linear for only a limited range, or "window," of endotoxin concentrations, a range that is usually less than 10-fold. The window can be shifted by choosing a different incubation time point. An endpoint assay is convenient for the quantitation of concentrations within a known 10-fold range.

With kinetic methodology, on the other hand, the OD is read at multiple time points as the reaction proceeds, with no termination step. The rate of the reaction is a function of the concentration of endotoxin. Derivation of the rate of the development of the turbidity reaction is complicated by the sigmoid shape of the reaction curve, and hence some form of mathematical transformation of the OD readings is required (73, 166, 351, 352, 385). Several less complicated approaches to estimating the rate of the reaction, such as the time to reach a given "threshold" OD reading (81, 185, 215), time to gelation (57), or the time taken between two predetermined OD readings (153), have been described.

A kinetic assay has several advantages over an endpoint assay in this type of analysis (250). The kinetic assay is able to quantify the concentration of endotoxin over a wider range, usually a 2- to 4-$\log_{10}$-fold range. Hence, a kinetic assay in a microtiter format is the most efficient and least operator-intensive method to quantify concentrations which extend over a range of more than 10-fold (81, 215). However, with the microtiter format, some loss of precision will occur because of timing errors in the addition of reagent to multiple wells of a 96-well plate and, with the repeated readings, inability to control the incubation temperature in the microtiter plate readers as commonly available in clinical laboratories. Under these conditions, intraassay coefficients of variation as high as 20 to 25% can be expected (159).

Another methodology in which the kinetics of chromogen conversion are monitored during the second step of the chromogenic LAL assay (55) is "pseudokinetic" because the period of monitoring does not correspond to the period of LAL activation. A single-step kinetic LAL method in which a colyophilized LAL and chromogenic reagents are used has been described. This assay is complicated by the differences in the reaction optima of the two reagents (81, 215).

Endotoxin Potency in the LAL Assay

The result of the LAL assay is obtained by comparing the activity of a known amount of endotoxin of known activity (the standard) with the activity of an unknown amount of endotoxin in the sample. Both endotoxin and the LAL reagent are subject to biological variation (392). Substantial variation in the reactivity of LAL from different sources has been observed (80, 86, 153), as has the LAL reactivity of endotoxins from different sources and a differential potency range as great as 200-fold (55, 87, 99, 103, 107, 140, 154, 195, 290, 342, 391, 392, 401, 402).

This variation in endotoxin potency is not limited to its in vitro activities (87, 103, 401). An extreme example of this variation in in vivo potency is the LPS derived from *Rhodopseudomonas sphaeroides*, which is not only less potent but in some systems is an endotoxin antagonist (307). Studies with human volunteers have established that the threshold pyrogenic doses for endotoxin of *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* are approximately 0.1, 1.0, and 60 ng/kg, respectively (123). To aid in the standardization of endotoxin testing, a U.S. Pharmacopeia Endotoxin Reference Standard by which endotoxin units are calibrated has been developed; its potency in rabbits and human volunteers has been carefully documented (148).

TABLE 1. Comparison of reactivity of various gram-negative organisms in LAL and rabbit pyrogen assays

Reference	Gram-negative bacterium	Ratio of reactivity in rabbit pyrogen assay to reactivity in LAL assays[a]
Wachtel and Tsuji (392)	*Escherichia coli*	35–52
	Salmonella typhi	
	Proteus mirabilis	
	Pseudomonas spp.	
Sveen et al. (362)	*Salmonella enteritidis*	4.3
	Veillonella spp. (2 strains)	84–896
	Fusobacterium spp. (2 strains)	
	Bacteroides spp. (3 strains)	
Wong et al. (410)	*Legionella pneumophila*	1,000
Weary et al. (402)		
Group 1	*Yersinia enterocolitica*	2–6
	Acinetobacter calcoaceticus	
	Salmonella abortus-equi	
	Shigella dysenteriae	
	Escherichia coli (3 strains)	
Group 2	*Vibrio cholerae*	26–75
	Serratia marcescens	
	Pseudomonas aeruginosa (2 strains)	
Devleeschouwer et al. (71)	*Escherichia coli*	1
	Klebsiella pneumoniae	9–12
	Serratia marcescens	10–11
	Pseudomonas aeruginosa	1–15
	Pseudomonas putida	15–500

[a] Ratio of dose of endotoxin (362, 392, 402) or bacteria (71, 410) required for reactivity with the rabbit pyrogen assay to dose for reactivity with the LAL assay.

Comparison with Other Bioassays

Three limitations applicable to all bioassays apart from the merely technical considerations are the questions of specificity and sensitivity and the problem of biological variation of both the assay and the substrate. The evidence for specificity has not been closely examined for endotoxin assays other than rabbit pyrogen and LAL assays (229).

The reactivity of endotoxin with LAL is merely one of several possible measures of its activity, although it is the most sensitive, being as much as 300 times more sensitive than the rabbit pyrogen test, for example (57, 289, 362, 392). In general, the results of endotoxin assays other than LAL (e.g., pyrogenicity in rabbits, mitogenicity, and lethality for mice and chicken embryos) parallel the LAL reactivity, at least for endotoxins of a limited range of gram-negative bacteria (87, 362, 379). An exception is endotoxin assays that reflect complement activation by LPS, as this may proceed through mechanisms independent of the lipid A component (87).

The difference in the reactivity of endotoxins in the LAL assay compared with other bioassays is more marked for endotoxins of diverse bacterial origin (Table 1). For example, endotoxins from environmental bacteria (287) such as *Legionella pneumophila* (410) or from anaerobic bacteria (362) are as much as 1,000 times more LAL reactive than are endotoxins derived from *E. coli* in concentrations that are equally pyrogenic in rabbits. That the rabbit pyrogen assay underestimates the activity of endotoxin from environmental bacteria in comparison with the LAL assay has great practical significance to pharmaceutical companies whose products are required to meet pyrogen-free standards.

A second example of differences in endotoxin activity as detected by different assays is that occurring as a consequence of the enzymatic modification of endotoxin. The neutrophil enzyme acyloxyacyl hydrolase, for example, leads to a selective deacylation by removing the secondary (acyloxyacyl-linked) acyl chains of lipid A but not the glucosamine-linked 3-hydroxyacyl chains (94), which results in a 100-fold reduction in dermal Shwartzman response but only a 10-fold reduction in the reactivity in the LAL assay (251).

Immunoassays

The contrasting properties of immunoassays versus bioassays for endotoxin are analogous to those differences between immunoassays versus bioassays for cytokines (405). In contrast to the LAL and other bioassays for endotoxin, immunoassays do not quantitate biological activity and are relatively insensitive, typically of the order of 10 ng of endotoxin per ml in plasma (194). Also, since reactivity is with the polysaccharide component of the LPS molecule, these assays are restricted by endotoxin immunotype. Various adaptations to overcome these obstacles have been described (194, 205, 272). For example, an immuno-*Limulus* assay in which a monoclonal antibody that is either type specific (243) or cross-reactive (333) is used to capture endotoxin onto a solid phase in microtiter plates and then the endotoxin is detected with the chromogenic LAL has been described.

Changes in the physical state of LPS, for example, as a consequence of disaggregation (348), result in changes in the biological activity of LPS, which is not proportionately reflected by changes in antibody binding in an immunoassay. Munford et al. (252, 253) examined the correlation between a solid-phase serotype-specific radioimmunoassay and the LAL assay, using two different types of LPS. With LPS purified by phenol extraction, quantitation by the two assays was essentially identical. In contrast, with LPS in CSF of rabbits with experimentally induced meningitis with *E. coli* of this serotype, the levels detected by radioimmunoassay were 10-fold higher than those detected by LAL assay.

In the serum of patients with meningococcemia, polysaccharide of *N. meningitidis* can be detected and quantitated by various methods, including counterimmunoelectrophoresis for group-specific polysaccharide and gas chromatography-mass spectrometry. High levels detected by counterimmunoelectrophoresis are associated with hypocomplementemia, thrombocytopenia, and hypotension (151, 211). In patients with meningococcemia, the levels of endotoxemia measured by the LAL assay correlate closely with the levels detected by gas chromatography-mass spectrometry (32).

Recently, a rapid whole-blood agglutination test (330b) has been developed for the detection of endotoxin, using a conjugated reagent that incorporates polymyxin B, a cyclic peptide antibiotic with high affinity for the conserved core region of endotoxin, conjugated to the Fab fragment of antibody 1C3/86. This antibody binds with high affinity to a site on glycophorin A present on erythrocytes from all human blood groups. This reagent has been evaluated in a preliminary trial with blood collected from patients diagnosed with endotoxemia with results comparable to those with the LAL assay (330a).

TABLE 2. Characteristics of various body fluids and seawater that affect the LAL assay

Fluid	Interference with LAL[a]	Frequency of infections due to gram-negative bacteria as a proportion of total	Observed correlations		Reference(s)
			Bacterial density (CFU/ml)	Endotoxin concn (ng/ml)	
Blood	++	Variable			
Urine	+	High	$<10^3$	<20	260, 351
			$>10^5$	200–300	
			10^6	10–30	165
			10^7	70–180	
CSF	−	Variable			
		(*H. influenzae*)	10^6	100	9
			10^7	500	
Bronchoalveolar lavage	−	Variable	10^4	0.5	305
Cervical and urethral exudates	?	High	NS[b]	10	295
Ascites	?	Variable			370
Synovial fluid	?	Variable			380
Seawater	−	NA[c]	10^5	0.4	398

[a] ++, Presence of inactivators of endotoxin or inhibitors of LAL; +, inhibitors of LAL only; −, no sample-related interference; ?, sample interference unknown.
[b] NS, not stated.
[c] NA, not applicable.

PRACTICAL CONSIDERATIONS

In general, for body fluids other than blood, three factors determine the clinical utility of the LAL assay: the density of bacteria found in clinically significant infections, the proportion of all infections at that site that are due to gram-negative bacteria, and the presence of interfering factors. These factors are summarized in Table 2.

In several fluids, there is a close correlation between concentrations of endotoxin and counts of bacteria over a broad range. As a consequence of this correlation, the LAL assay can be adapted to enable a rapid identification of specimens with amounts of gram-negative bacteria at clinically significant levels. For example, the finding of endotoxin above a particular breakpoint concentration in specimens of urine (184, 186, 187), vaginal fluid (295), and bronchoalveolar lavage fluid (305) can be used as a rapid method for the detection of urinary tract infections, bacterial vaginosis, and ventilator-associated gram-negative pneumonia, respectively. However, it should be noted that the exact quantitative correlation is variable for different genera of gram-negative bacteria (Fig. 6) (142, 165) and different fluids (Table 2).

Clinical Experience with LAL Assay for Fluids Other than Blood

Urine. With the number of gram-negative bacilli found in urinary tract infections, typically, at least 100,000 CFU/ml, the amount of endotoxin is well within the range of detection of the LAL assay. The inhibitory factors in urine are easily neutralized by dilution of the urine before performance of the LAL assay (339, 340). The positive predictive value for the presence of a gram-negative urinary tract infection is greater than 85%, and the negative predictive value is greater than 95% (259, 260, 262). Because at least 90% of uncomplicated urinary tract infections are due to gram-negative bacilli, a LAL test of urine is an effective screening procedure (184, 293), especially for high-risk groups such as pregnant women (259).

CSF. There is extensive experience with the LAL test as an aid to the detection of gram-negative meningitis (52, 83, 97, 188, 192, 236, 244, 261, 323, 380). In the detection of gram-negative meningitis, the LAL assay performs well in comparison with Gram stain, culture, and antigen detection tests, with a sensitivity and specificity of 97 and 99%, respectively, although sensitivity is decreased when bacterial counts are less than 50 CFU/ml, as in nosocomially acquired meningitis (332). CSF endotoxin levels may remain detectable for as long as 5 days (25) or, for *Haemophilus influenzae* meningitis, 9 days (188) after the initiation of appropriate antibiotic therapy.

Most, although not all (25), studies have found that levels of endotoxin correlate with measures of inflammation in the CSF (9, 35, 244, 257). The endotoxin level also has prognostic value in that levels in CSF of greater than 150 ng/ml are associated with seizures, levels of greater than 3,000 ng/ml correlate with systemic complications such as neutropenia, and levels of greater than 3.2×10^6 ng/ml correlate with a fatal outcome (82). The close correlation between CSF levels of endotoxin and bacterial counts could account for the apparent prognostic significance of endotoxin levels. For example, Feldman (98) also found an association between levels of endotoxin in the CSF and adverse outcome but considered this association to be an indirect reflection of the slower response to therapy that is associated with higher initial CSF bacterial counts (97).

Other fluids. Jorgensen (183) has reviewed the LAL assay experience with other fluids that have been studied. These include synovial fluid (380), cervical and urethral exudate fluid (132, 300–302, 354, 355, 420), ascitic fluid (370), peritoneal dialysate (51), ocular specimens (227, 409), amniotic fluid (321), and middle ear effusions (168). The LAL assay applied to fluids other than blood has been described as a useful supplement to conventional diagnostic techniques in that it is

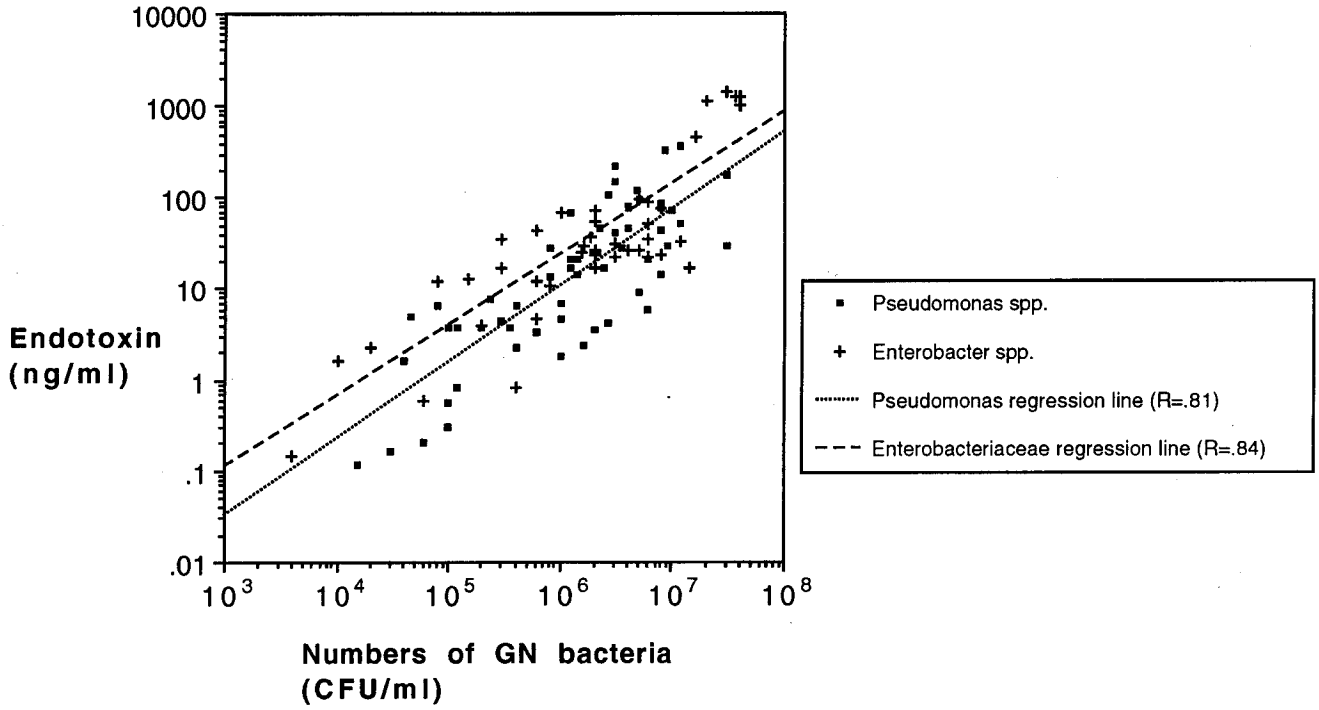

FIG. 6. Quantitative correlation between endotoxin concentration, by a modified kinetic LAL assay, and counts of viable bacteria per milliliter of infected urine. The concentration of endotoxin in urine infected with a member of the *Enterobacteriaceae* is two to three times higher for a given CFU count than in urine infected with a *Pseudomonas* species. GN, gram negative. Reprinted from reference 165 with permission of the publisher.

"a potent tool for rapidly revealing the presence of gram-negative microorganisms in a variety of body fluids" (183).

LAL Endotoxin Assay for Blood Samples

When the LAL assay is used to detect endotoxin in blood, two obstacles additional to those described for fluids other than blood are encountered: (i) the complex and poorly understood inhibitory factors present in blood, and (ii) the levels of endotoxemia generally being at the limit of test detection. Figure 7 illustrates the complex interaction among components of blood, endotoxin, and LAL. Endotoxin interacts with several components of plasma, including bile salts, proteins, and lipoproteins, leading to disaggregation, some inactivation, and the formation of complexes. These multiple effects of plasma on the activity of endotoxin are not always apparent as inactivation. Surprisingly, the lethal effect of endotoxin in rabbits can be enhanced by the coadministration of human plasma (20).

Inhibition by plasma and serum. The loss of reactivity to LAL on addition of endotoxin to plasma or serum is partly reversible, in that reactivity can be restored by dilution with distilled water or saline (210), and partly irreversible (180). The ability of plasma or serum to inhibit endotoxin activity is time dependent and temperature sensitive, being maximal at 37 to 45°C and abolished after plasma or serum is heated at 60°C for 5 min, and varies in proportion to the endotoxin potency. These characteristics imply an enzymatic inactivation of endotoxin by native plasma (180, 274, 275, 277, 283, 403), although this has yet to be definitively demonstrated.

Some workers have found that endotoxemic states tend to be associated with decreased endotoxin inactivation rates (199, 277, 279), although others have found increased inactivation activity in plasma from septic patients (283). In a panel of serum and plasma from normal donors, a 10- to 100-fold range

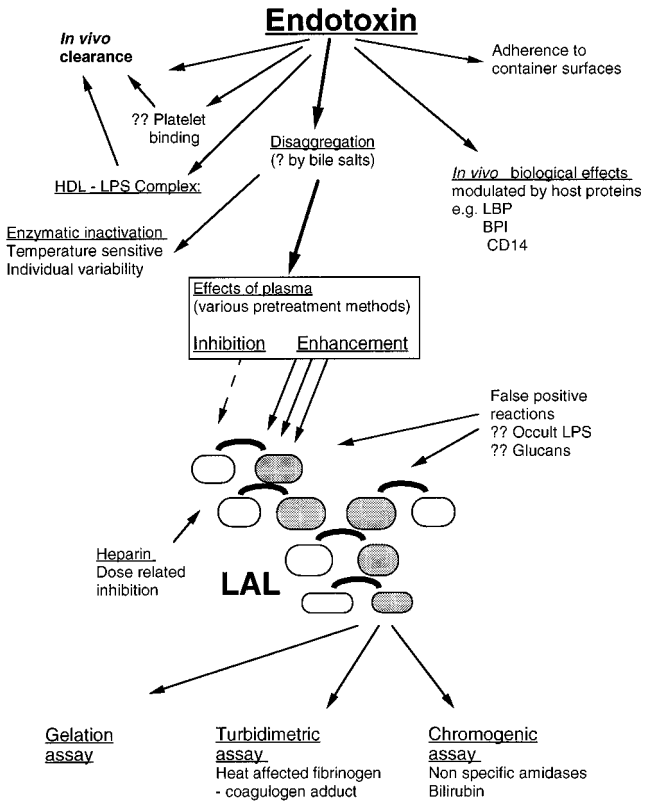

FIG. 7. Schematic overview of the factors that interfere with the detection of endotoxemia by the LAL assay. HDL, high-density lipoprotein; LBP, LPS-binding protein.

in the inhibitory effect of the endotoxin-LAL reaction can be found (24, 55, 275, 397). This large range in endotoxin neutralization activity is unrelated to differences in the immunoglobulin G and M antibody levels against the homologous endotoxin (101, 102, 397, 403) or the absolute levels of immunoglobulins in plasma (24). The inhibitory effect varied little in serum drawn from one individual 24 h apart (275). Some have found a correlation between the endotoxin-inactivating activity of human serum and the levels of lipoproteins (102).

(i) Serum versus plasma. In the LAL assay, less endotoxin can be detected in serum than in plasma obtained from whole blood to which endotoxin had been added (55, 73, 210, 277, 359, 365). The basis for this difference in recovery from plasma and serum is unclear. While some have found serum to have a higher endotoxin-inactivating activity than plasma (279), others have found the opposite (274).

(ii) Anticoagulants. Both heparin (359, 360) and another anticoagulant, CPD adenine-1 (235), exert a profound, dose-related inhibitory effect on the LAL assay. The inhibitory effect of heparin has been found at concentrations as low as 10 U/ml (359) and can result in a 90% reduction in apparent endotoxin concentrations at heparin concentrations of 30 U/ml (235). The inhibitory effect of heparin is mediated by the precipitation of LAL clotting enzyme proactivators (220).

(iii) Platelets. LPS can be detected in platelet thrombi formed in vivo by using immunohistochemical techniques (384). Therefore, it might be expected that endotoxin yields from platelet-rich plasma would be much higher than those from platelet-poor plasma as a consequence of endotoxin binding to platelets (69, 264, 331). However, recent reports have found no difference in the endotoxin recovery from platelet-rich or platelet-poor plasma (55, 108, 140, 265, 277, 279, 283, 359, 363).

False-positive reactions and enhancement of the LAL reaction by plasma. A false-positive LAL result occurs when the LAL reacts with a substance that is not endotoxin (or LPS or a component thereof), whereas the enhancement phenomenon occurs when the LAL reaction to endotoxin is enhanced by another substance or factor.

(i) False-positive reactions. Because the proclotting enzyme of the LAL shares several properties with mammalian coagulation factors, i.e., a requirement for calcium, inhibition by diisopropyl fluorophosphate, the presence of gamma-carboxyglutamic acid, and their activities as serine proteases, there is a theoretical possibility of activation of LAL by human coagulation factors. Activation of the coagulation cascade by a gram-negative bacterial infection, for example, typhoid (42), appears insufficient to lead to a positive LAL assay result. Positive LAL tests have been noted in plasma obtained from healthy volunteers during an infusion of fat emulsion. These results were associated with significantly increased levels of triglyceride chylomicrons and very low density lipoprotein (157).

The reagents used in the assay, such as distilled water, heparin, or normal saline, should be certified pyrogen-free reagents of a grade that would be suitable for administration to humans. A common problem leading to erroneous results is the use of commercially available heparinized blood collection tubes, which cannot be assumed to be either sterile or pyrogen-free (206, 313). The preparation of sterile tubes with measured amounts of sterile heparin at 20 U/ml is advised (159).

(ii) Enhancement phenomenon. Paradoxically, in addition to its endotoxin-inactivating activity, plasma also has an amplification effect on the reaction between LAL and endotoxin. The presence of heat-treated plasma results in more efficient gelation and a standard curve of OD against endotoxin concentration that is both elevated and steeper than the standard curve of the reaction conducted in distilled water alone (73, 140, 141, 166, 324, 385).

There are two mechanisms of the enhancement effect. The first is that a component of plasma enhances the development of turbidity in the LAL reaction. This may occur as a result of formation of an adduct between LAL coagulogen and heat-affected fibrinogen: a fibrinogen concentration of 2.5 g/liter in heated plasma can mimic an apparent concentration of endotoxin of 0.1 endotoxin unit per ml in the chromogenic LAL assay (270). This type of enhancement is most apparent in nonchromogenic turbidimetric LAL assays (166).

The second mechanism of enhancement is that a component of plasma facilitates the interaction of endotoxin and the LAL enzymes and leads to an amplification of the endotoxin activity. Because lipid A is lipophilic and forms complexes with high-density lipoproteins in plasma (383), it is postulated that the improved physical dispersion resulting from such binding may enhance the interaction with the LAL proenzyme and thus cause the apparent increase in yield in comparison to that in aqueous solution (140). This type of enhancement is most apparent in kinetic LAL assays (73, 166).

Because of these enhancement effects, which can result in a 1- to 2-$\log_{10}$-fold increase in apparent concentration, for LAL assays of plasma the standard endotoxin concentration curve should be determined in normal plasma that has been treated by the pretreatment method as for the samples (324), although few studies state whether this was done. It is not known whether the enhancement effect of plasma is constant from plasma taken from patients with different pathophysiological conditions (e.g., sepsis or liver disease) or indeed from different sites (portal venous or systemic venous).

(iii) Nonspecific amidolytic activity. The chromogenic LAL substrate is susceptible to cleavage by several amidases (e.g., plasmin, thrombin, and urokinase) found in blood (365). The treatment of plasma by dilution and heating (277, 403), but not chloroform (277), will inactivate the nonspecific amidolytic activity. With chromogenic assays, interference from the bilirubin pigment in plasma may be an important consideration in samples from jaundiced patients (171).

Specimen collection, handling, and storage. There is a tendency of endotoxin to adhere to surfaces of different containers, which is apparent by the variable recovery of endotoxin after desiccation of a solution containing endotoxin. There is substantially more adsorption onto polypropylene container surfaces than onto glass or polystyrene, for example (276). The importance for clinical samples of this loss of endotoxin through adsorption to the surface of specimen containers is unclear. The problem of adherence to container surfaces may be most relevant in the reconstitution of desiccated solutions of standards. Different types of reaction tubes may also affect the LAL reactivity with endotoxin (352). For example, borosilicate glass may increase the sensitivity of the reaction by 1 $\log_{10}$, although this effect is variable for borosilicate from different manufacturers (324). Endotoxins on the surface of glassware and plastics sterilized by autoclaving rather than by dry heat are not inactivated.

Because of the rapid inactivation of endotoxin in untreated plasma, these samples should be collected and processed on ice. In studies of stored samples, there was 30% loss of endotoxin in plasma stored at 4°C after 72 h, whereas no loss of endotoxin activity has been found with sample storage at −20°C for 2 weeks (283), −40°C for 3 months (140), or −70°C for 6 weeks (286).

Plasma pretreatment techniques. Several techniques have been described for the treatment of plasma samples to control the interfering substances in plasma before LAL assay. Six

TABLE 3. Clinical correlates of endotoxemia in settings other than sepsis[a]

Reference	Diagnosis or procedure	Endotoxemia			Correlate of endotoxemia
		No. of patients in whom endotoxemia was detected/total no. (%)	Mean (pg/ml)	Range (pg/ml)[b]	
Brocke-Utne et al. (39)	81-km marathon run	72/89 (81)	NS[c]	<100–>1,000	Association with race length, nausea and vomiting
Gordon et al. (121)	Maintenance hemodialysis	7/24 (29)	NS	<10–50	Limited association with pyrogenic reaction
Casey et al. (49)	Cardiopulmonary bypass (pediatric)	16/24 (67)	NS	<0.1–438	No association with perioperative morbidity
Exley et al. (95)	Pancreatitis (severe)	12/14 (32)	314	<25–5,000	Association with severe disease and nonsurvival
Exley et al. (95)	Pancreatitis (mild)	7/24 (29)	<25	<25–5,000	
Guarner et al. (131)	Cirrhosis	45/51 (88)	86	<27–224	Association with renal dysfunction
Kokuba et al. (199)	Cirrhosis	NS/57 (ns)	10	<2–40	No correlation with severity of liver dysfunction; negative correlation with plasma fibronectin

[a] Representative studies selected from >50 studies that applied the LAL test to patients or subjects as given in the text. When stated, the methods used in the studies in this table were the chromogenic version of the LAL assay together with the pretreatment of plasma by dilution and heating.
[b] Lower limit of the range is usually the detection limit as given for each study.
[c] NS, not stated.

methods are now largely of historic interest: chloroform extraction (210), gel filtration (153), solid-phase binding (139), pH shift (314), perchloric acid (366), and dilution (213).

Dilution followed by heating has become the method of choice for the pretreatment of plasma because of its simplicity and superior efficacy compared with earlier methods (23, 55, 80, 111, 139, 140, 171, 239, 274, 277, 294, 324, 359). The optimal conditions have been studied by several groups, with a range of recommendations reported (38, 55, 108, 111, 140, 171, 239, 277, 294, 324, 359, 375, 403). The most critical parameter appears to be the inactivation temperature, which should not be below 60°C (108, 294, 324). A typical set of inactivation conditions is a 1:10 dilution of plasma in distilled water with heating at 70°C for 10 min.

CLINICAL CORRELATES OF ENDOTOXEMIA

A limited number of large recent studies which presented quantitative endotoxemia data using broadly comparable methods are summarized in Tables 3 and 4. The clinical studies of endotoxemia can be grouped into two broad categories: those in which endotoxemia has been detected in the absence of sepsis (Table 3), and those in which endotoxemia has been detected in patients at increased risk of sepsis (Table 4). Some conditions, such as liver disease and cirrhosis, have elements of both categories.

In early evaluations in the LAL test to detect endotoxemia, the unexpected finding of positive results in patients without sepsis, as in ambulatory patients receiving radiotherapy (86), and the unexpected finding of negative results in patients with sepsis and gram-negative bacteremia (222, 358) were cited as major failures of the LAL test. These unexpected results have been repeated in subsequent studies. However, to a large extent, they probably represent failings in our understanding of the significance of endotoxemia, its complex clearance properties, and the potential for host tolerance of its effects, rather than a failing of the LAL test.

Endotoxin Pharmacokinetics

Given the quantitative relationship between levels of endotoxin and counts of bacteria in fluids other than blood, a similar

TABLE 4. Clinical correlates of endotoxemia in patients with suspected or documented sepsis

Reference[a]	Diagnosis or procedure	Endotoxemia			Correlate of endotoxemia
		No. of patients in whom endotoxemia was detected/total no. (%)	Mean (pg/ml)	Range (pg/ml)[b]	
Brandtzaeg et al. (34)	Systemic meningococcal disease	24/45 (53)	NS[c]	<25–170,000	Correlation with complications (e.g., ARDS and renal dysfunction)
van Deventer et al. (390)	Suspected bacteremia	31/473 (6)	NS	<6–>100	Association with development of septicemia
Parsons et al. (286)	With ARDS	15/23 (64)	380	<10–3,000	Endotoxemia persistent in 56%
Parsons et al. (286)	At risk for ARDS	12/56 (22)	60	<10–90	Association with development of ARDS
Danner et al. (67)	Septic shock	43/100 (43)	440	<10–NS	Association with ARDS and renal dysfunction
Casey et al. (48)	Suspected sepsis	87/97 (89)	1,560	<60–7,500	No association with nonsurvival

[a] Representative studies selected from over 50 studies that applied the LAL test to patients or subjects as given in the text. The methods used, when stated, in the studies in this table were the chromogenic version of the LAL assay together with the pretreatment of plasma by dilution and heating.
[b] Lower limit of the range is usually the detection limit as given for each study.
[c] NS, not stated.

quantitative relationship between levels of bacteremia and endotoxemia might be expected. Limited data derived from experimental sepsis models suggest such a relationship (60, 347). Indeed, it has been noted that even in a carefully controlled animal model of sepsis with a uniform bacterial inoculum (266) the levels of endotoxemia were >10 times higher in nonsurvivors than in survivors ($P < 0.05$), and the differences in the levels of bacteremia paralleled the differences in levels of endotoxemia (>10-fold increase in nonsurvivors compared with survivors; $P < 0.01$). However, the data on which to assess the possible quantitative relationship between levels of endotoxemia and gram-negative bacteremia from clinical studies are very limited and inconclusive (34, 44, 358).

The amount of endotoxin associated with a single gram-negative bacterium is 30 to 40 fg ($\times 10^{-15}$ g) (268, 398). Assuming an origin from bacteria within the intravascular compartment, it would be expected that a positive LAL assay at a detection limit of 10 pg/ml would represent the detection of an amount of endotoxin equivalent to that within 300 gram-negative organisms per ml. This estimate does not take account of endotoxin clearance mechanisms, which are known to be extremely efficient. Indeed, administration to human volunteers of 2 ng of endotoxin per kg, a dose sufficient to induce host cytokine (64) and hematological (389) responses resembling those seen in sepsis and lasting for 2 to 4 h, resulted in a peak level of 12 pg/ml at 15 min which was undetectable (<3 pg/ml) within 30 min (389).

In patients with sepsis, endotoxemia is typically at levels as high as 400 pg/ml or higher (Table 4), although much greater levels are seen in patients with meningococcemia (34). Hence, it would be difficult to account for the positive results in patients with gram-negative bacteremia on the basis of the numbers of bacteria present, typically less than 10 CFU/ml in adults (415). Moreover, the unexpected finding of endotoxemia in patients without sepsis, which typically can be at concentrations as high as 200 pg/ml or higher (Table 3), is difficult to reconcile with the endotoxin load that this appears to represent.

Some recent observations in a controlled canine model of bacteremic septic shock suggest that the severity of sepsis pathophysiology cannot be inferred from the quantitative level of endotoxemia. Sepsis was induced by exposure to bacteria containing 26 (*P. aeruginosa*) (68) or 0 (*Staphylococcus aureus*) (265) fg of endotoxin per bacterium, respectively, in a comparison with sepsis induced by *E. coli* (25 fg per bacterium). The levels of endotoxemia were >20 times higher following challenge with *E. coli* than following that with *P. aeruginosa* ($P < 0.0001$) (68), whereas with *S. aureus* challenge, the levels of endotoxemia were negligible (265). By contrast, the associated manifestations of septic shock were either indistinguishable (265) or more severe (68) than the manifestations observed with *E. coli* challenge.

Moreover, it is misleading to attempt to extrapolate from a level of endotoxemia in a patient with sepsis to an exogenous dose of endotoxin that would induce comparable pathophysiological abnormalities. Exposure to a bolus dose of endotoxin, as in "intoxication" experimental models, results in a burst of cytokine production at high levels, in contrast to the low-grade ongoing cytokine production observed in experimental models of infection or even in clinically observed gram-negative sepsis (64). Episodes of inadvertent transfusion of blood contaminated with psychrophilic gram-negative bacteria suggest a complex relationship between the amount of endotoxin infused (as volume of contaminated blood) and the patient cytokine response (226) or survival (357).

Endotoxin Clearance

Bacterial endotoxin is known to interact with numerous components of blood, including erythrocytes, mononuclear cells, platelets, neutrophils, lipoproteins, and plasma proteins (324a). Clearance of endotoxemia is effected both by humoral inactivation and through uptake into liver and mononuclear phagocyte cells (47, 69, 106, 350), and it is influenced by both host- and LPS-specific factors. There is a complex relationship among binding of endotoxin to antibodies, to platelets, and to lipoproteins in the modulation of its biological effects and clearance (138, 212). Binding of endotoxin by chylomicrons probably facilitates the clearance of endotoxin through the liver (312). By contrast, the binding to high-density lipoprotein results in the formation of a complex (high-density lipoprotein–LPS) which is cleared more slowly than free LPS (half-lives of several hours versus several minutes, respectively) and more slowly even than native high-density lipoprotein (383).

The clearance patterns (115) and biological effects (64) following administration of live bacteria are different from those following the administration of an equivalent dose of purified endotoxin. Also, the clearance and tissue distribution of live bacteria are delayed in rabbits concurrently infused with endotoxin (198).

In several clinical studies, dysfunction of these clearance systems may have contributed to the maintenance of the endotoxemia, which may explain its association with thrombocytopenia (69), neutropenia, and hepatobiliary dysfunction (47). In patients with gram-negative bacteremia, there is an association among endotoxemia, the severity of underlying disease, and low opsonic titers against the homologous bacterial isolate (421).

Endotoxin Tolerance

Tolerance to the pyrogenic and other effects of endotoxin has been studied with experimental animals and human volunteers (181). Observations of patients in the preantibiotic era, when endotoxin was administered for therapeutic purposes to patients with neurosyphilis (143), indicate that a profound state of endotoxin tolerance can be induced. For example, a typical course of daily endotoxin injections would start at doses equivalent to 100 million killed typhoid bacteria and would increase to 50 billion over the following 10 days (143).

The tolerance to endotoxin in humans resembles that seen in experimental animals in that it appears to be mediated by two components: an early-appearing transient cellular refractory state and a late-appearing humoral component that assists the clearance of endotoxin by the mononuclear phagocyte system (127–129, 181).

Early tolerance can be demonstrated as the absence or attenuation of the pyrogen response which is apparent at 24 to 48 h following a single large dose of endotoxin. This type of tolerance is short-lived and is limited to endotoxins and not to other pyrogens. It appears to result from the temporary refractoriness of effector cells to produce endogenous pyrogen and other endogenous mediators in response to endotoxin.

Late tolerance induced by repeated daily injections of endotoxin appears by about the third or fourth day and subsides within 3 to 4 days after injections are stopped. This form of tolerance is less potent than that achieved with early-phase tolerance and is greatest to the homologous inducing endotoxin but does extend to other endotoxins. It appears to be mediated at least in part by serotype-specific antibodies and also through binding to lipoproteins (396).

Tolerance to endotoxin has been found in patients in the convalescent phase of typhoid fever and tularemia (125, 269),

pyelonephritis (228), and chronic *Salmonella* bacteremia (204). Tolerance may persist for as long as 4 months after clearance of bacteremia (204). These observations stimulated a series of experiments involving rabbits and several hundred human volunteers to define the mechanisms of tolerance in the context of typhoid fever in humans (124, 155, 181, 247). There were three surprising observations in these studies. Within 2 weeks of convalescence from either typhoid fever or tularemia, a high degree of tolerance of the early-phase type could be demonstrated. Further studies demonstrated that the mechanisms underlying a state of tolerance that had been induced by prior intravenous administration of endotoxin remained functional during symptomatic typhoid fever or tularemia, and yet the severity of the clinical illness in tolerant volunteers was indistinguishable from that in nontolerant volunteers (126).

These observations present a paradox. On the one hand, the acquisition of tolerance during the course of a typhoid illness suggests a substantial systemic exposure to endotoxin associated with the infection. On the other hand, the observation that the course of typhoid in endotoxin-tolerant volunteers is no different than in naive volunteers casts doubt that the ongoing symptoms of typhoid can be attributable to endotoxin released from bacteria into the circulation. This led to the concept of the compartmentalization of the host response to account for the dissociation between systemic and local tissue reactivity in that the latter could persist and mediate the symptoms in the face of systemic tolerance.

It is unclear how these observations with typhoid relate to other types of gram-negative sepsis. It has subsequently been observed that endotoxemia is indeed infrequent in typhoid patients regardless of symptoms (42, 219, 238). By contrast, the response to *Brucella* endotoxin is exaggerated in patients with active brucellosis in comparison to control nonimmune subjects (1).

Observations in patients with systemic meningococcal disease illustrate the extent to which the host response to sepsis may be compartmentalized. Patients with meningococcemia have much higher levels of endotoxin and cytokine production in the plasma compartment than in the CSF, whereas in patients with meningitis, the reverse is noted (33, 35).

Endotoxemia without Sepsis

Liver disease. There are >20 studies of endotoxemia in the context of liver disease (12, 31, 76, 109, 110, 119, 131, 170, 199, 218, 304, 369, 377), liver transplantation (27, 30, 134, 240, 245, 417), or pancreatic disease (65, 95, 105, 408). Endotoxemia has long been suspected of having pathogenic properties in patients with liver disease even in the absence of overt gram-negative sepsis (271). The origin of the endotoxin in this setting is also believed to be from the gastrointestinal tract because several studies have found a portal-to-systemic gradient of endotoxin levels, with higher levels in portal venous blood than in peripheral blood (26, 177, 218, 304). There has been some success at reducing endotoxemia by the oral administration of endotoxin-binding agents such as bile salts (376), colistin (131), and paromomycin (369), although not with a selective decontamination regimen (27) or laxatives (364). This endotoxemia in part also reflects an impaired ability to eliminate endotoxin through the liver, which may be compounded by the effects of alcohol excess (31), and an impaired ability of plasma from cirrhotic patients to inactivate endotoxin (199).

The significance of endotoxemia in patients with liver disease is unclear. Some studies have found an association between endotoxemia and abnormalities in routine biochemical liver function tests (26), whereas others have not (109, 199, 304), although it should be noted that these tests are relatively insensitive indicators of liver dysfunction in comparison to histological evidence (110). Some workers have found endotoxemia to be more common in patients with alcoholic than nonalcoholic cirrhosis (31, 109). Also, several studies have found an association of endotoxemia with complications of liver disease such as renal dysfunction (12, 46, 131, 218, 369, 377), encephalopathy (26, 59) or postoperative morbidity (170).

In liver transplant patients, two studies from one center have found an association of endotoxemia with adverse events, including graft nonsurvival (245, 417), whereas three other studies have not found this association (27, 30, 134). Two studies identified the cadaveric liver as the potential source of endotoxin (293a, 417).

Hemodialysis. Pyrogenic reactions are an important problem with hemodialysis, and there is concern that this is due to contamination of the dialysis water with bacteria or endotoxin (292, 310) or contamination resulting from the use of reprocessed dialyzers (100, 122). There is uncertainty as to whether endotoxin is able to cross the different types of dialyzer membranes and also whether the LAL-reactive material (LAL-RM) found in the plasma of patients undergoing hemodialysis is something other than endotoxin.

The evidence that endotoxin and other pyrogens are able to cross at least some types of dialysis membranes has been reviewed by Lonnemann (216). Interestingly, the ability of endotoxin to cross the dialysis membrane is enhanced by the presence of plasma on the opposite side of the membrane. It is suggested that the LAL-RM is a cellulose-based material, possibly (1-3)-β-D-glucans, which has properties distinct from endotoxin (322) and reacts with the factor G-drive pathway of LAL (424). An endotoxin-specific assay which does not react with (1-3)-β-D-glucans has been developed and applied in this context (368). On the other hand, other workers have found that the LAL-RM is polymyxin B inhibitable (241) and that levels of LAL-RM in plasma parallel evidence of contamination in the dialysate (144, 400), properties that would implicate endotoxin as being the LAL-RM.

In any event, LAL testing of plasma of hemodialysis patients has limited ability to detect pyrogenic reactions, having positive and negative predictive values of less than 70% (121).

Intestinal endotoxemia. An origin from the gastrointestinal tract has often been presumed for endotoxemia in patients with gastrointestinal diseases (58, 404) and also in patients receiving radiotherapy to the abdomen in association with symptoms of nausea (225). Marathon runners with marked symptoms of nausea (39) and racehorses (13) completing races have levels of endotoxemia similar to those seen in the radiotherapy patients. Of interest, salmonella antigens have been found by immunostaining in the joint material of patients with reactive arthritis following salmonella infection. The presence of salmonella LPS in the absence of viable organisms is thought to be pathogenetically important in reactive arthritis (122a).

Other conditions. The occurrence of endotoxemia and even bacteremia (329) from the gastrointestinal tract as a consequence of hemorrhagic shock is disrupted. In four small studies of patients at high risk for adult respiratory distress syndrome (ARDS) following trauma, endotoxemia was not found in any patient (77, 93, 147) even when samplings of portal blood were obtained (246). Other studies have shown that, surprisingly, endotoxemia may even precede the biochemical evidence of intestinal permeability in patients with hemorrhagic shock (328) and that administration of endotoxin to human volun-

teers results in biochemical evidence of increased intestinal permeability (282).

Transient endotoxemia occurs in patients undergoing minimally invasive procedures of the urinary (114, 318, 367), biliary (217), or gastrointestinal (190) tract. In general, the severity of symptoms and the degree or frequency of detection of endotoxemia in these patients are higher when gram-negative bacteria are found at the sites of these procedures. In premature neonates, there is an association between endotoxin in cord blood and growth of gram-negative bacteria from placental samples (335).

There are 11 studies of endotoxemia in patients undergoing cardiopulmonary bypass (6, 7, 29, 49, 179, 364), major vascular surgery (11, 327, 353), and extracorporeal membrane oxygenation (145). Indeed, endotoxemia increases during splanchnic ischemia in association with the period of aortic cross clamping that occurs during major vascular surgery, implicating the gastrointestinal tract as a source of endotoxemia (179, 319, 327, 353). Another source of endotoxin is autologous blood collected intraoperatively for retransfusion which becomes contaminated during the salvage process (29). The consequences of this endotoxemia are unclear. Even in the two largest series with 24 (49) and 38 (29) patients, respectively, there was no association with postoperative morbidity.

Endotoxemia in Patients at Increased Risk for Sepsis

There have been more than 50 studies of the use of the LAL test to detect endotoxemia in patient groups in which there was an increased risk of sepsis as a consequence of neutropenia, burns, or various pediatric conditions or when patients met the criteria of suspected or documented sepsis. The LAL test has also been used successfully as an epidemiological tool in identifying a gram-negative bacterium as the causative agent in a newly identified disease process (37).

Concordance of endotoxemia with gram-negative bacteremia. Gram-negative bacteremia has often been used as a standard for comparison with the result of the assay for endotoxin (288, 374). However, the use of gram-negative bacteremia as a basis for comparison should be cautioned for two reasons. From the earliest studies with LAL (208, 209) and even earlier studies using the rabbit bioassay (21, 78, 237, 297), it has been apparent that there is a poor concordance between gram-negative bacteremia and endotoxemia (163). Endotoxemia is detected in approximately half or less of those with gram-negative bacteremia, and similarly, gram-negative bacteremia is detected in approximately half of those with endotoxemia (163).

It cannot be assumed that endotoxemia and gram-negative bacteremia are interdependent phenomena. Circulating antigenemia in the absence of bacteremia has been found in other settings, for example, pneumococcal disease (16, 193). Endotoxemia is often intermittent in patients with gram-negative sepsis (67, 231, 311). Moreover, the type of organism causing bacteremia may be at least as important a factor in this concordance as the limit of sensitivity of the assay (163). In published comparisons of endotoxemia and gram-negative bacteremia, the association is more common when the blood isolate is not a member of the family *Enterobacteriaceae* than when it does belong to this family.

Discordance between the LAL test and blood culture results may occur for various technical reasons. Following the initiation of antibiotic therapy, endotoxin may be undetectable within a period as short as 12 h for typhoid bacteremia (219) and 36 h for meningococcemia (34), or as long as 10 days for plague (43, 44) and 20 days for leptospirosis (399). On the other hand, false-negative and false-positive test results as a consequence of inappropriate collection procedures are a problem. False-positive results for endotoxin resulting from contamination may occur as often as 9% of the time even with expert collection (412).

A second limitation in using bacteremia as a basis for comparison with endotoxemia is that gram-negative bacteremia is itself a relatively weak predictor of clinical outcome (356), being apparent only in those studies large enough to enable a stratification of the patients into different categories of illness, different age groups, different types of pathogens (202), or different grades of bacteremia (415). Among a group of patients with systemic sepsis, patients with gram-negative bacteremia cannot be prospectively identified by the use of simple clinical criteria (291). Endotoxemia is somewhat more predictive of clinical outcome (41, 390). In a study of 473 patients of whom 31 were found to have endotoxemia, 53 were found to have gram-negative bacteremia and 17 were found to have both. The positive predictive value for the subsequent development of clinical septicemia was higher for the detection of endotoxemia (positive predictive value = 48%) than for the detection of gram-negative bacteremia (positive predictive value = 28%) (390).

Prognostic significance of endotoxemia. The conclusions drawn in each study of endotoxemia as an indicator of disease severity and prognosis have varied. In some studies, there is a direct quantitative correlation between the level of endotoxemia and frequency of adverse events and mortality as in studies of patients with meningococcemia (34), plague (44), and leptospirosis (399), whereas studies with several hundred patients (67, 390) with suspected gram-negative sepsis have found a merely qualitative rather than a quantitative association with adverse outcome; yet, equally large studies (86, 358) have not found any association between endotoxemia and outcome, either qualitative or quantitative. Two recent studies found that the levels of endotoxemia in patients with sepsis syndrome were predictive of outcome only if they either were incorporated into an LPS-cytokine score that also included levels of tumor necrosis factor alpha, interleukin-1β, and interleukin-6 (48) or were associated with elevated levels of these cytokines (91). A third study has also found a complex relationship among the levels of endotoxin, elevated levels of these cytokines, and patient survival (75).

The reasons for the disparate conclusions from the different studies are complex. In general, the direct quantitative correlation between level of endotoxemia and disease severity was apparent in studies of a single infection type, as in an epidemic setting, whereas in studies showing only a qualitative association, the patient groups had heterogeneous types of infection and much lower frequencies of documented gram-negative infections. The copresence of gram-negative bacteremia with endotoxemia appears to be an important determinant of prognosis (163c).

In part, some of the disparate results from different studies may reflect the use of less sensitive methodologies. For example, the clearly quantitative relationship between levels of endotoxemia and disease severity in meningococcemia was apparent in studies that used the chromogenic LAL assay with the dilution and heat method of plasma treatment (34) and not in earlier studies (211, 382) that used the gelation LAL assay with the chloroform method of plasma treatment. The latter method is as much as 100-fold less sensitive than the currently used dilution and heat extraction method (324).

(i) Neutropenia. As a correlate of the presence of fever or nonsurvival, two studies (19, 418) have shown an association with levels of endotoxemia in patients with neutropenia,

whereas three studies with fewer patients have not (196, 232, 343). In one study, elevated levels of LPS which failed to decline were predictive of death from uncontrolled infection (19). A study in bone marrow transplant recipients who were neutropenic because of their chemotherapy and radiotherapy showed an association between biochemical evidence of increased gut permeability, which was a consequence of the therapies and graft-versus-host damage, and endotoxemia (176).

(ii) Neonates and children. In two studies of malnourished children, endotoxemia was associated with nonsurvival in one (280) but not the other (197). Scheifele and coworkers have shown an association between endotoxemia and thrombocytopenia with necrotizing enterocolitis (336–338). They also found endotoxemia to be surprisingly common (36 of 219) in samples of routinely collected cord blood from neonates at birth (335) and in premature neonates following the initiation of enteral nutrition (337). Complications of shigellosis, such as hemolysis and renal failure, were more common in children with endotoxemia than in those without it (201). In the patients with shigellosis, the endotoxemia was found more commonly in younger children (<2 years) and always preceded the onset of hemolysis, by as much as 6 days. Endotoxemia has also been detected in children with cystic fibrosis (371) and neonates following abdominal surgery (169) and pediatric patients with suspected sepsis (96).

(iii) Burns. In different studies of patients with burn injuries, endotoxemia has been found to correlate with the sepsis score (255), the size of the burn injury (92, 407), colonization of the burn with gram-negative bacteria (182), and nonsurvival (74, 92). Approaches to reduce levels of endotoxin in this setting have included polymyxin (255, 256), immunoglobulin supplementation (254), and vaccination (182).

(iv) Suspected or documented sepsis. Several large studies, each with at least 20 patients, have examined the relationship between endotoxemia and ARDS in patients either with documented gram-negative sepsis (34) or at risk for gram-negative sepsis (27, 67, 133, 286, 412). ARDS is relatively common in this setting; it is often refractory to conventional therapies, including appropriate therapy for gram-negative sepsis; and on the basis of extensive animal model data, it is suspected to be at least in part mediated by endotoxin (221). An association of endotoxin with the development of ARDS in patients with sepsis has been found in three studies (34, 67, 286) but not in three other studies (27, 133, 412).

In 40 patients at risk for nosocomial infection as a consequence of being intubated, a patient group in which the gastrointestinal tract has been thought to be a potential reservoir for gram-negative bacteria and endotoxin, plasma levels of endotoxin did not correlate with counts of gram-negative bacteria in the stomach (315). In typhoid (3) and gram-negative sepsis (311), some have found levels of endotoxemia to correlate with liver dysfunction. In leptospirosis (399), the level of endotoxemia correlates with renal dysfunction, although this may be a reflection of the specific localization of this infectious process to the kidney.

ENDOTOXIN-SPECIFIC THERAPIES

Endotoxin is one of a multitude of mediators of sepsis that are under consideration as potential targets for novel therapies for sepsis. Other mediators, for example, tumor necrosis factor, interleukin-1 and platelet-activating factor, and thromboxane A_2, have been the subject of recent reviews (54, 117). Endotoxin is a particularly attractive therapeutic target in sepsis (61) and has been the subject of extensive preclinical studies. It appears to be the most powerful and broadest-spectrum stimulus to the sepsis cascade mechanisms both as an initiator and as a potentiator. Moreover, it has a conserved structure with a biosynthetic pathway that is unique to gram-negative bacteria. It appears to serve a key structural role within the gram-negative bacterium. As a consequence, several agents with specific binding and inhibitory activity against endotoxin have been developed.

TABLE 5. Anti-endotoxin-based therapeutic strategies

Strategy and approach	Reference(s)[a]
Reduced production or release	
KDO[b] synthesis inhibitors	120, 135, 136
Antibiotics	160, 163a, 344
Reduce adsorption	
Bile salts	46, 376
Whole-gut irrigation, laxatives	77, 364, 404
Colistin, paromomycin	131, 369
Selective decontamination	27
Accelerated clearance	
Extracorporeal	8, 28, 36, 388
Immunoglobulin supplementation	19, 130, 176, 255, 298, 334
Immunotherapy	
Polyclonal	2
Monoclonal	412
Antagonists	
Polymyxin	45, 170, 256, 257, 320
BPI and N-terminal fragments	284
Lipid X	66

[a] Studies that have evaluated the effects of therapy on endotoxemia.
[b] KDO, 3-deoxy-D-manno-2-octulosonate.

Table 5 lists various strategies proposed for the therapy of gram-negative sepsis that target endotoxin either directly or indirectly. The rationale for their development and the results of preclinical studies are discussed elsewhere (61–63, 162). Not all of them have been evaluated in clinical trials, and of those that have, it is difficult to evaluate their efficacy as few of the studies were randomized, blinded, or even controlled. Because of the complex nature of gram-negative sepsis and its therapy, the enrollment of several hundred patients is required for a study to show a conclusive survival benefit for any new therapeutic agent.

The enthusiasm for the inhibition of endotoxin activity should be tempered by the recognition that despite numerous agents with promising results in preclinical studies from over 20 years of research, no anti-endotoxin approach to the therapy of septic shock has emerged (62). Many of the preclinical studies have used animal models of endotoxin intoxication which understate the complex nature of endotoxin in gram-negative sepsis (64, 162). In these intoxication models, all of the effects observed can be attributed to "endotoxication." Whether this is the case for clinical gram-negative sepsis can be questioned (161). Improvement in response to anti-endotoxin therapies is not consistently related to changes in the levels of endotoxemia in different experimental models (163b). Moreover, endotoxin-induced inflammation is not always detrimental in that it may serve as a critical host defense to infections arising at mucosal sites (214, 281, 361).

Strategies for Therapy

3-Deoxy-D-manno-2-octulosonate synthesis inhibitors. The 3-deoxy-D-manno-2-octulosonate biosynthetic pathway leading

to formation of lipid A is an attractive target for inhibition (135, 136, 309). 3-Deoxy-D-manno-2-octulosonate synthesis inhibitors have antibacterial activity in vitro and in vivo (135, 136) and greatly enhance the susceptibility of gram-negative bacteria to serum-mediated killing (120).

Antibiotic-induced release of endotoxin and the JHR. In the design of therapeutic strategies for gram-negative sepsis, there is the concern that antibiotics neutralize the bacteria but not the endotoxin released as a consequence of antibiotic action (344). A widely cited syndrome in this context is the Jarisch-Herxheimer reaction (JHR). The JHR is a striking syndrome in which patients with various conditions such as syphilis (345), tick-borne relapsing fever (156), leptospirosis (399), and louse-borne relapsing fever (112, 267) exhibit transient pyrexia, hypotension, and rigors as early as 2 h after the first dose of antibiotic. The nature of the bacterial mediator of JHR is elusive, with evidence both for (40, 112, 116, 413) and against (137, 308, 345, 419) a role for endotoxin.

In settings other than the JHR, there is clear evidence that antibiotics increase the bioavailability of endotoxin from gram-negative bacteria by as much as 20 times the preantibiotic concentrations (9, 164, 346). This leads to speculation that the propensity of antibiotics to release endotoxin should be a consideration in their selection for therapy of gram-negative sepsis (344). While there is suggestive evidence, the clinical importance of this effect remains to be determined (160). Some have suggested that alteration in the bacterial cell surface following the action of antibiotics may enhance the efficacy of antibodies to core region epitopes of LPS in gram-negative bacteria (285, 349).

Polymyxin. Polymyxin and other polycationic molecules (320) bind to lipid A in a stoichiometric ratio. The perfusion of plasma over a column to which polymyxin is bound is an effective method for removing endotoxin from the plasma of septic rats (53) and also septic patients (8, 45). When systemically administered in an animal model, polymyxin can moderate some of the deleterious effects of overwhelming gram-negative sepsis, such as hypotension, independently of changes in levels of endotoxemia, which may remain similar to (104) or be unexpectedly higher than (14) that in control animals.

In clinical studies, polymyxin has been evaluated in patients with obstructive jaundice without apparent effect on endotoxemia or mortality (170). In an uncontrolled study, a threefold reduction in endotoxin levels (76 to 21 pg/ml) was observed in 16 patients with sepsis and multiple organ failure treated with direct hemoperfusion using a polymyxin B column (8).

Plasmapheresis. Removal of endotoxin through plasmapheresis or whole-blood exchange has been attempted in selected settings such as meningococcemia and septic neonates. These procedures are hazardous in patients with severe septic shock with hypotension and disseminated intravascular coagulation. Hemorrhage from sites of vascular access has contributed to severe or fatal sequelae in two series (36, 388).

Monoclonal and polyclonal immunotherapy. The core region of LPS is highly conserved among gram-negative bacteria that are serologically distinct on the basis of O-polysaccharide antigen specificities (146). The rationale for anti-endotoxin immunotherapy is based on the expectation that this structural conservation implies that antibodies reactive to this core region should be cross-protective against heterologous challenge.

Early observations, both experimental and clinical, were supportive of this rationale (reviewed in reference 427). Antibodies to the LPS core region were shown to be protective against lethal challenge with either heterologous endotoxin or live gram-negative bacteria in experimental models. The levels of anti-core region antibody in the sera of patients with gram-negative bacteremia correlated with enhanced survival either independently of or in addition to the correlation with type-specific antibody (230, 296, 429). Administration of human immune serum to patients with gram-negative sepsis resulted in a substantial reduction in mortality in a randomized controlled trial (428).

However, the results of more recent clinical trials with polyclonal and monoclonal (233a) anti-endotoxin antibodies have been equivocal (reviewed in references 17, 62, 63, and 162). Some have been unable to reproduce the preclinical studies demonstrating anti-endotoxin activity, and the mechanism of protection has been the subject of intense speculation and investigation (18, 50, 54, 394, 411). There is now doubt that the ability of these antibodies to neutralize endotoxin activity in the LAL assay is predictive of their protective activity in an in vivo challenge with endotoxin (63, 234, 393) or could account for the results observed in the clinical trials (18). In a canine model of gram-negative sepsis, the effect of different anti-LPS antibodies, whether to increase (152) or decrease (306) survival, cannot be explained simply on the basis of the effects on levels of endotoxemia. In these animal models, anti-endotoxin antibodies and antibiotics are synergistically protective in comparison to either therapy alone (56, 285).

Antagonists. Studies with synthetic (307, 317) and naturally occurring (307) lipid A molecules raise the possibility that partial structures that might retain some of the beneficial effects of endotoxin, such as the immunostimulatory properties, without the harmful effects (e.g., induction of hypotension and activation of coagulation) or could even act as antagonists of lipid A could be developed. Lipid X, an antagonist of lipid A, has been evaluated in an animal model of gram-negative sepsis and unexpectedly was found to significantly decrease survival (66). Monophosphoryl lipid A has been used as an adjuvant to enhance the immune response to malaria circumsporozoite protein vaccine (315a).

Endotoxemia and Response to Therapy

The LAL assay has been used to evaluate the effects of these therapies on the levels of endotoxemia and also as a basis for selection of patients to receive anti-endotoxin therapies (2, 19).

In the two largest double-blind trials, contrasting results were obtained. In a subset of patients from the HA-1A anti-endotoxin monoclonal antibody trial, endotoxemia remained detectable at 24 h after therapy in a similar proportion of patients receiving either HA-1A (4 of 12) or placebo (4 of 9; $P = 0.673$). Of the patients who were endotoxemic at study entry, there was a significant difference in mortality favoring HA-1A recipients (5 of 16 HA-1A versus 8 of 11 controls; $P = 0.034$) (412). By contrast, in a trial of polymyxin therapy in early burn injury patients, the levels of endotoxemia were significantly and markedly reduced in polymyxin recipients, whereas there was no difference in either morbidity, as reflected in a sepsis score, or mortality in comparison to the control group (255).

In a smaller randomized trial of oral paromomycin, an aminoglycoside antibiotic, in 24 patients with cirrhosis, clearance of endotoxemia was associated with improved renal function in recipients of paromomycin in comparison to placebo recipients (369).

In three studies of commercially available immunoglobulin preparations, significant differences in the levels or frequency of detection of endotoxemia were noted (130, 298, 334). Differences in morbidity and mortality were apparent in one study with 55 patients (334) but not in an earlier trial with 46 patients (130). In the third study in 63 bone marrow transplant recipients, a reduction in infection-related mortality was noted (298).

Serial quantitative measurements of endotoxemia have been used to estimate its clearance half-life in meningococcemic patients. In patients treated with plasma exchange, the half-life was approximately 180 min (388) versus approximately 120 min in 15 conventionally treated meningococcemic patients from another study (34).

CONCLUSIONS

The complex biological properties of both endotoxin and the LAL reagent have been greatly clarified as a result of numerous studies in the last 10 to 15 years. In a range of body fluids other than blood, the detection of endotoxin with the LAL can be used as an aid to identify the presence of gram-negative bacteria. Several modifications to the assay have been made in an attempt to allow the quantitative measurement of endotoxin and also to improve its application to blood samples.

The most simple and efficient method for the control of the inhibitory factors in plasma is the method of dilution and heating. While the quantitation of endotoxin can be made by using endpoint methods, the limitations of this technique should be recognized.

The most complex aspect in the detection of endotoxemia, whether qualitative or quantitative, is the interpretation of the result. In general, the clinical significance of the finding of endotoxemia broadly parallels the frequency and importance of gram-negative sepsis in the patient groups studied. However, comparisons of quantitative results obtained with different reagents, different assay runs, different types of gram-negative infection, and different patient groups are complex. While LPS levels decline in parallel to the response to effective therapy, it is unclear that clinical improvement can be accelerated by therapies designed to antagonize endotoxin.

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