JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1994, p. 2120–2127
0095-1137/94/$04.00+0

Vol. 32, No. 9

Concordance of Endotoxemia with Gram-Negative Bacteremia in Patients with Gram-Negative Sepsis: a Meta-Analysis

JAMES C. HURLEY*

Division of Infectious Diseases, Children's Hospital & Medical Center, Seattle, Washington 98105-0371

Received 8 April 1994/Returned for modification 12 May 1994/Accepted 7 June 1994

The *Limulus* amebocyte lysate (LAL) assay is a sensitive method for detecting endotoxin. Using gram-negative (GN) bacteremia as the basis for comparison, concordance with endotoxemia in 45 studies could be expressed as an odds ratio. Calculation of summary odds ratios by the Mantel-Haenszel-Peto method indicated that the concordance of the results was no higher by the chromogenic LAL assay than by the gelation version, and the sensitivity was improved by only 11% (62 versus 51%). Endotoxemia was detected in 77 (68%) of 114 patients with bacteremia caused by an organism that was not a member of the family *Enterobacteriaceae*, whereas endotoxemia was detected in only 120 (45%) of 269 patients with bacteremia caused by a member of the family *Enterobacteriaceae* or an anaerobe ($P < 0.001$). This difference was also apparent for patients with GN bacteremia for whom a fatal outcome had been recorded. The prevalence of GN bacteremia in the tested population and the type of etiological agent are critical and previously unrecognized variables which affect the interpretation of the LAL test in patients with suspected sepsis.

Endotoxin is a cell wall component found exclusively in gram-negative (GN) bacteria. Considerable experience has accumulated with the use of the *Limulus* amebocyte lysate (LAL) for the detection of endotoxin in various body fluids (20). For example, when applied to samples of cerebrospinal fluid for the detection of GN meningitis, the LAL assay has a sensitivity and a specificity each greater than 95%, in comparison with the sensitivity of Gram stain examination, which is only 81% (58).

By contrast, its value as a diagnostic test when applied to plasma samples remains unclear, despite more than 45 studies. With the detection of GN bacteremia as the basis for comparison, its sensitivity and specificity are less than 85% (20). Several estimates made on the basis of experimental (65) and clinical (8, 11, 64) data suggest that the minimum amount of endotoxin required to induce a positive LAL test result would be equivalent to that contained in at least 100 GN bacteria per ml, a level that is seldom reached in patients with clinical bacteremia. In addition, plasma contains incompletely defined factors that interfere with the detection of endotoxin (32, 41, 57) or that confound the reading of the assay (32).

To overcome these obstacles, the assay has been modified. In 1983, a chromogenic substrate (CLAL) was introduced as a more sensitive indicator of LAL activation by endotoxin. The CLAL assay is sensitive to endotoxin in plasma samples spiked in vitro at concentrations 10 times lower than those used in the previous gelation end point (GLAL) assay (28). However, there has been no direct comparative study of the two assays on samples from patients with GN bacteremia.

Additional considerations have come to light more recently. It has long been assumed, and implied in the term itself, that endotoxin is released only on death and lysis of the bacterial cell. Recently, it has been recognized that endotoxin is shed from the surface of certain GN bacteria in the absence of lysis and that this property is not equally expressed by different GN bacteria (62). For example, it is observed more often with invasive than with mucosal isolates of *Neisseria meningitidis* (2, 49).

A third consideration relates to the patient population. The proportion of patients with GN bacteremia is a key indicator of the patient mix in studies which may or may not have been designed for the purpose of examining the diagnostic application of the assay. In large published series of studies of patients satisfying generally accepted criteria of suspected GN sepsis, this proportion is usually in the range of 13 to 40%. For example, in five such studies, the proportions were 13% (22), 25% (53), 30% (6), 37% (74), and 40% (71).

The purpose of this review is to examine the diagnostic experience with endotoxemia detection by using GN bacteremia as the basis for comparison. The value of the more sensitive CLAL assay, the etiological agents of the bacteremias, and the prevalence of GN bacteremia in the patient population were three factors identified for special attention. The statistical techniques of meta-analysis are used. These techniques evolved from their application to the evaluation of retrospective studies of disease (24a, 45a).

MATERIALS AND METHODS

Study selection. A review of the literature was performed. The review included a search of the Medline database back to 1972, with the search headings being "septicemia" (prior to 1992), "bacteremia" (from 1992 on), "endotoxins," and "human." The search was not limited to reports published in the English language (13, 34) and included conference proceedings (14, 19, 48, 69). This was supplemented with a manual search of the references from each report retrieved, review articles, and textbooks. The following criteria were used in selecting studies for inclusion: (i) study design, which included a direct comparison of blood culture and LAL (or *Tachypleus* amebocyte lysate [67]) assay methods applied to blood from patients suspected of having GN sepsis; (ii) size, with at least four patients studied; and (iii) data presentation, such that each patient could be classified into one of four possible categories: category 1, blood cultures, GN bacteria, and endotoxin positive; category 2, blood cultures, GN bacteria, and endotoxin negative; category 3, blood cultures, no GN bacteria,

* Corresponding author. Mailing address: Division of Infectious Diseases, Children's Hospital & Medical Center, P.O. Box 5371/CH 32, Seattle, WA 98105-0371. Phone: (206) 528-2116. Fax: (206) 527-3890. Electronic mail address: jhurley@u.washington.edu.

and endotoxin positive; and category 4, blood cultures, no GN bacteria, and endotoxin negative.

Unpublished data were sought directly from the authors of studies who had published results in a form which did not meet the above criteria to seek a clarification that would have enabled inclusion. The exclusion criteria were duplicate publication and application of the assay to patients with conditions other than suspected sepsis. Twenty-eight studies were excluded (the list is available on request). For one study (33), only the data from the examination of systemic blood were included and the data for portal blood examination were excluded.

Data analysis. For each study, the concordance of the results of the two tests was expressed as an odds ratio (OR), which was calculated by using the formulae given in the Appendix (calculation of study-specific ORs).

To enable a comparison of the studies, I used the meta-analysis procedure of Yusuf et al. (73). Details of the calculations are given in the appendix (calculation of summary ORs). In brief, for each study (i), the expected number (E_i) of endotoxin-positive patients among those in whom GN bacteremia was detected was calculated, assuming statistical independence from the marginal totals in a two-by-two table. The observed number (O_i) of endotoxin-positive patients minus the expected number ($O_i - E_i$) and its variance (V_i) were calculated. The values of $O_i - E_i$ and V_i for each study were summed to produce a grand total (GT) and total variance (VT), respectively. From these totals, the summary ORs and their 95% confidence intervals (95% CIs) were calculated.

Data aggregation and sensitivity analysis. I began the analysis with all of the studies divided into two groups on the basis of which of the two versions of the assay, GLAL or CLAL, was used. A subgroup analysis was conducted by using only studies in which the proportion of patients with GN bacteremia was in the range of 13 to 40% (as in several large series [6, 22, 53, 71, 74]), after it was found that many of the studies in which this proportion was outside of this range were designed for purposes other than to examine the use of LAL as a diagnostic test for unscreened patients with suspected sepsis.

The homogeneity of the concordance among the studies in each analysis was tested as described by Yusuf et al. (73) (see Appendix [calculation of summary ORs]). A finding of significant heterogeneity indicates that the variation in concordance among studies is more than could be expected from random variation. Summary estimates may be inappropriate in cases of significant heterogeneity.

Diagnostic utility. For each analysis, a calculation of sensitivity, specificity, and positive predictive value (PPV) was derived from the formulae as given in the Appendix (calculation of assay parameters).

RESULTS

Forty-five studies were identified; those studies reported the results for a total of 2,539 patients with suspected GN sepsis. There were only 43 separate publications because two publications (45, 52) each provided the results of two studies. A total of 29 studies used the GLAL assay (Table 1) and 16 studies used the CLAL assay (Table 2). The concordance between the detection of endotoxemia and GN bacteremia in these studies is illustrated in Fig. 1.

In 24 studies sepsis was suspected on the basis of fever and clinical assessment. In nine studies blood for culture and plasma were drawn from patients with suspected sepsis in the settings of specific illnesses such as malnutrition (37, 50), pancreatitis (23), and obstructive jaundice (4, 44) or following abdominal surgery (25, 26, 33) or liver transplantation (5). Nine studies were limited to patients with specific infections such as typhoid fever (1, 9, 45, 48), salmonellosis (45), plaque (10, 11), or meningococcemia (8, 29). These patients were usually selected on the basis of a clinical diagnosis supplemented by confirmatory microbiological testing either by a positive culture for the specific pathogen from any site or by serology. One study (69) included only patients with microbiologically documented urological sepsis. The sensitivity limit for GLAL studies was usually in the range of 0.5 to 10 ng/ml, and that for CLAL studies was in the range of 10 to 500 pg/ml. This variability in sensitivity limits reflects the nonuniform potency among both the different reference endotoxin standards and the LAL reagents used in each study. In addition, the method of plasma pretreatment was more commonly by the dilution and heating method in the CLAL studies than in the GLAL studies (10 of 16 versus 7 of 29 studies).

Diagnostic utility. As an aggregate result, of the 2,539 patients from the 45 studies, GN bacteremia was detected in 615 (24%) of them. However, there was a large range in the pretest probability for GN bacteremia in individual studies (4 to 86%). Moreover, the derivation of a summary OR incorporates a formal test for heterogeneity which was highly significant in the two series of studies (Table 1 and 2).

Of 10 studies in which the prevalence of GN bacteremia was 50% or greater, microbiological documentation of GN sepsis by serology or cultural isolation of the organism from blood or another site was either an elective or an obligate requirement for patient inclusion in 7 studies (1, 8, 9, 11, 45, 48, 69). Of eight studies in which the prevalence of GN bacteremia was 10% or less, five (4, 5, 25, 33, 44) were studies of endotoxemia occurring in the setting of obstructive jaundice or abdominal surgery.

Comparison of the summary results for studies that used the CLAL assay versus those that used the GLAL assay gave similar results for concordance (6.3, 4.4 to 9.1, and 8.4, 5.9 to 11.8, respectively [values are summary ORs, 95% CIs]), sensitivity (54 and 53%, respectively), and PPV (40 and 49%, respectively). Further comparison of studies that used the CLAL assay versus those that used the GLAL assay was limited to the 22 studies (9 used the CLAL assay and 13 used the GLAL assay) in which the percentage of patients having GN bacteremia in each study was in the range 13 to 40%. In the 9 studies that used the CLAL assay, the assay sensitivity was 62% (74 of 119 patients) and the PPV was 35% (74 of 214 patients). Overall, in the 13 studies that used the GLAL assay, the assay sensitivity was 51% (82 of 161 patients) and the PPV was 50% (82 of 163 patients). The summary ORs (with 95% CIs) indicate that the concordance with GN bacteremia was significantly less for the CLAL assay (3.6, 2.3 to 5.6) than for the GLAL assay (20.1, 12.5 to 32.4). Recalculation of the chi-square test for heterogeneity yielded results for the two series that were not statistically significant, a finding that is consistent with the absence of heterogeneity.

Bacteriology. The identifications of the isolates that caused bacteremia in 387 patients from 34 studies were tallied (Table 3). Endotoxemia was detected in 79 (68%) of 116 patients with bacteremia caused by an organism that was not a member of the family *Enterobacteriaceae* (non-*Enterobacteriaceae*), whereas endotoxemia was detected in only 120 (45%) of 269 patients with bacteremia caused by a member of the family *Enterobacteriaceae* or an anaerobe (chi-square, 16.9; $P < 0.001$; degrees of freedom = 1; Table 3). This difference was also apparent for patients with GN bacteremias in whom a fatal outcome had been recorded; endotoxemia was found in 26 of

TABLE 1. Endotoxemia in the presence or absence of GN bacteremia in studies that used the GLAL assay

Author(s), yr (reference no.), and diagnosis[a]	Assay		No. of patients in whom endotoxemia was detected/total no. of patients (%) in whom GN bacteremia was or was not detected		% GN bacteremia[b]	Excluded[c]
	Sensitivity limit[d]	Codes[e]	Detected	Not detected		
Levin et al., 1970 (40), Q	5	Ch	10/15	7/83	15	
Levin et al., 1972 (39), Q	5	Ch	17/31	19/187	14	
Martinez-G et al., 1973 (46), Q	5	pH	1/16	1/67	19	
Das et al., 1973 (17), Q	10	Ch	9/11	7/43	20	
Butler et al., 1973 (10), Pl	0.5	Ch	2/2	7/8	20	
Stumacher et al., 1973 (65), Q	0.3	Ch	28/65	26/74	47	X
Fossard and Kakkar, 1974 (25) SS	1	Ch	2/2	22/23	8	X
Oberle et al., 1974 (50), N	0.5	Ch	3/3	6/20	13	
Feldman and Pearson, 1974 (24), Q	1	pH	2/36	0/53	40	
Jirillo et al., 1977 (34), Q	1	Ch	1/2	4/8	20	
Bailey, 1976 (4), J	5	Ch	1/1	12/23	4	X
Butler et al., 1976 (11), Pl	1	Ch	3/5	0/5	50	X
Magliulo et al., 1976 (45), T	1	pH	8/12	1/2	86	X
Magliulo et al., 1976 (45), S	1	pH	4/4	9/21	16	
Clumeck et al., 1977 (13), Q	3	pH	9/12	2/36	25	
Jacob et al., 1977 (33), SS	1	DH	1/1	3/23	4	X
Butler et al., 1978 (9), T	1	Ch	0/13	0/8	62	X
Usawattanakul et al., 1979 (67), Q	0.5	Ch	34/40	2/18	70	X
Scheifele et al., 1981 (59), Q	10	DH	5/8	10/55	13	
Kelsey et al., 1982 (36), Q	?	pH	1/2	0/28	7	X
Togari et al., 1983 (66), Q	0.5	pH	1/1	7/9	10	X
McCartney et al., 1983 (47), Q	0.1	Ch	15/15	16/16	48	X
Cooperstock and Riegle, 1985 (14), Q	1	DH	10/11	6/32	26	
Scheifele et al., 1985 (60), Q	0.2	DH	3/5	20/42	11	X
Pearson et al. (GLAL), 1985 (52), Q	0.1	DH	5/5	0/2	71	X
Adinolfi et al., 1987 (1), T	0.3	DH	7/14	2/7	67	X
Klein et al., 1988 (37), N	5	Ch	0/1	7/15	6	X
Shenep et al., 1988 (64), Q	0.01	DH	9/10	3/16	38	
McGladdery et al., 1993 (48), T	0.04	?	0/16	0/6	72	X
All GLAL studies (n = 29)			191/359 (53)	199/930 (21)	28	
Excluding X (n = 13)			82/161 (51)	81/629 (13)	20	

[a] Diagnosis codes; Pl, plague; M, meningococcemia; Q, query sepsis; N, malnutrition; U, urosepsis; L, liver transplantation; T, typhoid; S, salmonellosis; P, pancreatitis; J, obstructive jaundice; SS postsurgical sepsis. For all studies that used the GLAL assay, chi-square for heterogeneity was 74.7 (degrees of freedom = 28; P = 1.0). Excluding X, chi-square for heterogeneity was 9.8 (degrees of freedom = 12; P = 0.37).

[b] Percentage of patients in whom GN bacteremia was detected.

[c] Excluded indicates studies in which the proportion of patients with GN bacteremia was outside the range of 13 to 40%.

[d] Sensitivity limit of the LAL assay; (data are in nanograms per milliliter except those for the study of McGladdery et al. (48), which are in endotoxin in units per milliliter.

[e] Codes for method of plasma or serum pretreatment: Ch, chloroform-phenol extraction; pH, pH shift; DH, Dilution and heat treatment; PCA, perchloric acid treatment; ?, not stated.

26 (100%) patients with fatal bacteremia caused by a non-*Enterobacteriaceae*, whereas it was found in 13 of 22 (59%) patients with fatal bacteremia caused by an *Enterobacteriaceae* (chi-square, 13.1; $P < 0.001$; degrees of freedom = 1).

The more frequent association of endotoxemia with bacteremia for non-*Enterobacteriaceae* versus *Enterobacteriaceae* was evident with the isolates stratified by type of test. That is, by the CLAL assay endotoxemia was found in 49 of 65 (75%) versus 28 of 47 (60%) patients with bacteremia caused by non-*Enterobacteriaceae* versus *Enterobacteriaceae*, and by the GLAL assay endotoxemia was found in 30 of 51 (59%) versus 92 of 222 (41%) patients with bacteremia caused by non-*Enterobacteriaceae* versus *Enterobacteriaceae*, respectively. Bacteremias caused by *Yersinia pestis* and *Salmonella* species were exceptions among the *Enterobacteriaceae*, being more commonly found in association with endotoxemia than without endotoxemia.

DISCUSSION

I caution that this analysis does not attempt to establish the clinical significance of a positive LAL test result in any given patient group but, rather, attempts to examine how close the concordance with GN bacteremia in the published experience is. Were endotoxemia concordant with GN bacteremia, this might be relevant to the application of new therapeutic modalities for sepsis (31, 63).

The 45 studies were heterogeneous with respect to diagnoses, patient demographics, and pretest probability of the patients having GN bacteremia, which limits any generalizations that can be made on the basis of the full set of studies. This inconsistency, which is flagged by the finding of a statistically significant test for heterogeneity, would not have been so readily appreciated from a review of the studies in the traditional style without the statistical techniques of meta-analysis.

TABLE 2. Endotoxemia in the presence or absence of GN bacteremia in studies that used the CLAL assay

Author(s), yr, (reference no.), and diagnosis[a]	Assay		No. of patients in whom endotoxemia was detected/ total no. of patients (%) in whom GN bacteremia was or was not detected		% GN bacteremia[b]	Excluded[c]
	Sensitivity limit (ng/ml)	Codes[d]	Detected	Not detected		
Harthug et al., 1983 (29), M	0.5	pH	2/2	1/3	40	
Pearson et al. (CLAL) 1985 (52), Q	0.1	DH	4/5	2/5	50	X
Hass et al., 1986 (30), Q	0.01	PCA	3/3	8/33	8	X
Dolan et al., 1987 (19), Q	0.01	DH	7/19	28/134	13	
Van Deventer et al., 1987 (69), U	0.005	DH	15/38	0/38	50	X
Van Deventer et al., 1988 (68), Q[e]	0.005	DH	17/53	14/420	11	X
Brandtzaeg et al., 1989 (8), M	0.025	DH	24/35	0/7	84	X
Lumsden et al., 1989 (44), J	0.015	DH	1/1	13/20	5	X
Függer et al., 90 (26), SS	1	Ch	5/5	17/19	21	
Danner et al., 1991 (15), Q	0.01	DH	11/19	32/81	19	
Dofferhoff et al., 1992 (18), Q	0.005	?	4/6	6/12	33	
Wortel et al., 1992 (71), Q	0.005	DH	18/32	9/50	39	
Exley et al., 1992 (23), P	0.025	DH	2/2	5/6	25	
Van Dissel et al., 1993 (70), Q	0.001	?	4/4	7/10	29	
Yoshida et al., 1994 (72), Q	0.003	?	21/30	35/106	22	
Bion et al., 1994 (5), L	0.02	?	1/2	31/50	4	X
All CLAL studies (n = 16)			139/256 (54)	208/994 (21)	20	
Excluding X (n = 9)			74/119 (62)	140/421 (33)	22	

[a] For all studies that used the CLAL assay, chi-square for heterogeneity was 39.8 (degrees of freedom = 15; P = 1.0). Excluding X, chi-square for heterogeneity was 3.5 (degrees of freedom = 8; P = 0.10). The diagnosis codes are as defined in footnote a of Table 1.

[b] See footnote b of Table 1.

[c] See footnote c of Table 1.

[d] See footnote e of Table 1 for definitions of abbreviations.

[e] Data from the study by van Deventer et al. (68) were obtained by backcalculation from summary data in the original article.

With any diagnostic test, caution is required in its interpretation in patient populations in whom the pretest probability of the disease of interest is unusually high or low. A positive test for the occurrence of endotoxin in patients with a low frequency of GN bacteremia may represent "intestinal endotoxemia," as in the studies of patients with obstructive jaundice or undergoing surgical procedures (4, 5, 33, 44), or a false-positive result because of ex vivo contamination, which may occur at rates as high as 9% even with experienced personnel (71). The clinical significance of intestinal endotoxemia, which may reach levels that are seen in patients with acute, potentially lethal infections, is unclear because these increases are associated with hemodynamic changes that are often clinically trivial and not predictive of any subsequent morbidity (3, 12, 56). On the other hand, many studies with a rate of GN bacteremia greater than 50% examined patient populations that had been screened to include patients with specific types of sepsis.

The presumption that, in patients with GN sepsis, GN bacteremia and endotoxemia must always be associated with each other, even at low levels, in part contributed to earlier criticisms of the LAL assay (21, 65) and stimulated the development of a more sensitive version. If this presumption is correct, then a more sensitive assay should substantially increase the sensitivity for the detection of GN bacteremia in patients with sepsis. However, this was not found to be the case because the sensitivity in the 16 studies that used the CLAL assay (54%) was only 1% higher than that in the 29 studies that used the GLAL assay (53%).

Even after excluding the studies that had patients with an unusually high or low prevalence of GN bacteremia, the CLAL assay showed an increase in sensitivity of only 11% (62 versus 51%) but a substantial decrease in specificity and PPV in comparison with those of the GLAL assay. Recalculation of the test for heterogeneity yielded a result consistent with its absence, although this should not be taken as evidence that the studies were truly homogeneous (27). Indeed, there were both a higher proportion of bacteremias caused by non-*Enterobacteriaceae* reported in studies that used the CLAL assay (65 of 112) than was the case for studies that used the GLAL assay (51 of 273) and also a more common use of the dilution and heating method of plasma pretreatment, a method considered to be more efficient than the chloroform technique (28, 32a). These differences could presumably account for some of the increase in sensitivity in the studies that used the CLAL assay.

What are the practical implications? In a hypothetical population of 100 patients with suspected sepsis for whom cultures of blood would be required, of which 20 have GN bacteremia, the GLAL assay would be positive for 10 of these GN bacteremic patients and produce discordant positive results for 10 patients without GN bacteremia. The CLAL assay, on the other hand, would be positive for 12 of the GN bacteremic patients but would generate discordant positive results for 24 patients.

An important determinant of the utility of the assay, which this analysis revealed, is the type of GN bacteremia being sought. For example, endotoxemia is found in association with only 32% of bacteremias caused by *Enterobacter* species but is found in association with 73% of meningococcemias. A study by Porter et al. (54), which was excluded from the present analysis because a rabbit bioassay for endotoxin was used to detect endotoxin in serum, also failed to detect endotoxemia in five patients with bacteremia caused by an *Enterobacteriaceae*; for three of these patients the outcome was fatal. Opal (51) found that endotoxin levels in patients with bacteremia caused by *Pseudomonas* species, meningococci, and the nonfermenta-

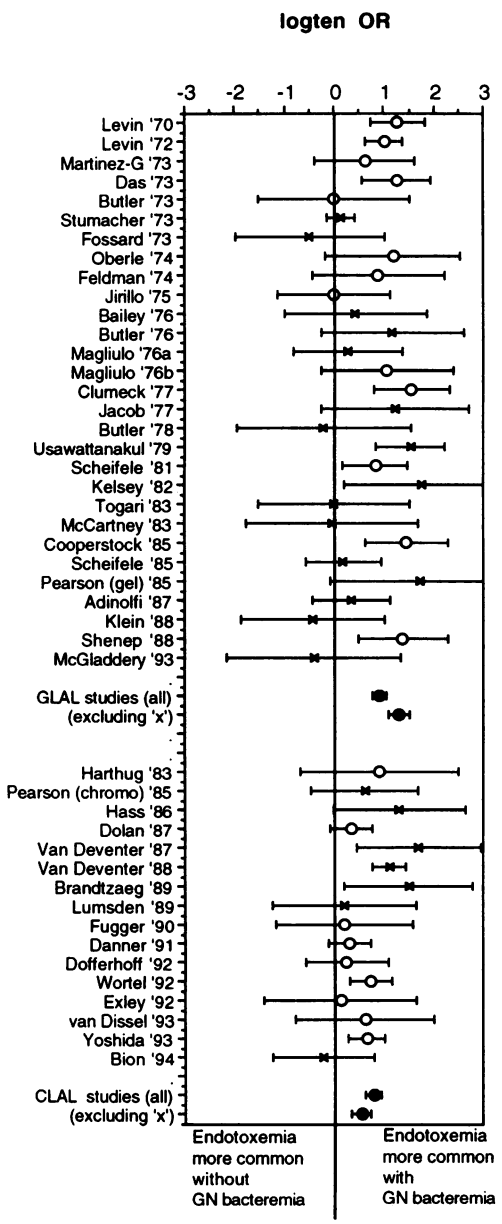

FIG. 1. Concordance between the detection of endotoxemia and GN bacteremia in 45 studies of patients with suspected GN sepsis expressed as study-specific (open symbols and crosses) and group summary (closed symbols) ORs and 95% CIs. Studies which used the GLAL version of the LAL assay are grouped separately from those that used the CLAL version. Studies in which the incidence of GN bacteremia was outside the range of 13 to 40% are indicated by a cross.

tive GN organisms tended to be higher than in patients with bacteremias caused by *Escherichia coli*, *Klebsiella* species, and *Enterobacter* species.

Because the intravascular compartment is seldomly the primary site of infection, mechanisms which enable GN bacteria and endotoxin to persist at detectable levels in this compartment, such as serum resistance and plasma lipoprotein binding (7, 43), respectively, warrant closer scrutiny in the interpretation of the LAL assay.

Nearly all GN bacteria isolated from the blood of patients are resistant to killing by normal serum, whereas one-third or

TABLE 3. Identification of GN bacteremias[a]

Organism	No. (%) of patients			
	All cases of GN bacteremias in which *endotoxemia* was:		Cases of GN bacteremias with fatal outcome in which endotoxemia was:	
	Present	Absent	Present	Absent
Members of the family *Enterobacteriaceae* and anaerobes	120 (45)	149 (55)	13 (59%)	9 (41)
Escherichia coli	38	44	6	2
Klebsiella sp.	21	23	3	1
Enterobacter sp.	8	17	3	1
Proteus sp.	7	9		
Serratia sp.	4	2		1
Anaerobes and others[b]	11	9		2
Salmonella typhi	16	40		
Salmonella sp.	10	3		2
Yersinia pestis	5	2	1	
Non-*Enterobacteriaceae*	79 (68)	37 (32)	26 (100)	0 (0)
Pseudomonas aeruginosa	35	23	6	
Xanthomonas and others[c]	9	1	9	
Neisseria meningitidis	30	11	11	
Haemophilus influenzae	5			
Neisseria gonorrhoeae		2		
All	199 (52)	186 (48)	39 (81)	9 (19)

[a] The data in Table 3 were compiled from previously published data (1, 4, 5, 8, 9–11, 13–15, 18, 23–25, 29, 30, 33, 34, 36, 37, 39, 40, 44–46, 48, 50, 60, 64–66, 68, 70, 72), including three provided as personal communications (15, 48, 72).
[b] Others include enteric GN rods and *Bacteroides* sp.
[c] Others include *Acinetobacter* sp., *Aeromonas* sp., *Pseudomonas cepacia*, *Flavobacterium meningosepticum*, and *Pseudomonas putida*.

fewer of the GN bacteria isolated from mucosal surfaces are susceptible (55), although the mechanisms of serum resistance vary (35, 38, 61). It is plausible that the ability of particular invasive bacteria to shed endotoxin from their surfaces, as is found with the systemic *N. meningitidis* isolates (2, 49), is a property associated with resistance to serum. As a consequence, it would be expected that bacteremias with these organisms will be associated with the copresence of shed endotoxin in quantities large enough to be easily detected in plasma, even by electron microscopy (7). By contrast, with *Enterobacteriaceae* such as the K-1 strain of *E. coli*, the resistance to serum is dependent on the coating of the cell with a minimum amount of capsular polysaccharide without a change in lipopolysaccharide expression (42). It would be expected that shedding of lipopolysaccharide would not be critical to the intravascular survival of these organisms.

Also, endotoxin is cleared from the peripheral circulation with a half-life of as short as 2 h in patients with fulminant meningococcemia (8), although longer half-lives might be expected in patients with infections in which the levels of acute-phase proteins, such as lipopolysaccharide-binding protein, are elevated.

The variable detection of endotoxemia in the setting of fatal cases of sepsis caused by different types of GN bacteria is analogous to similar observations in experimental models, which have caused some to question the role of endotoxin as the universal mediator of GN sepsis (16). The bacteriology and prevalence of GN bacteremia in the patient populations studied are two critical variables that should be carefully considered in future evaluations of that assay.

TABLE A1. Format to which the selected studies conformed

GN bacteremia	Endotoxemia[a]	
	Positive	Negative
Positive	a_i	b_i
Negative	c_i	d_i

[a] a_i, number of true positives; b_i, number of discordant negatives; c_i, number of discordant positives; d_i, number of true negatives.

APPENDIX

Selection of studies. Studies which conform to the format shown in Table A1 were included.

Calculation of study-specific ORs. The concordance of the two test results is expressed as an OR, where

$$\text{OR} = \frac{(a_i + 0.5) \times (d_i + 0.5)}{(b_i + 0.5) \times (c_i + 0.5)}$$

with

$$95\ \text{CI} = \frac{1.96}{\sqrt{\left(\frac{1}{a_i + 0.5}\right) + \left(\frac{1}{b_i + 0.5}\right) + \left(\frac{1}{c_i + 0.5}\right) + \left(\frac{1}{d_i + 0.5}\right)}}$$

Calculation of summary ORs. Summary ORs were calculated from all studies by the Peto modification of the Mantel Haenszel method (45a), where

$$\ln \text{OR} = \frac{\Sigma\,(O_i - \text{E}_i)}{\Sigma V_i}$$

with

$$95\%\ \text{CI} = \frac{1.96}{\sqrt{\Sigma V_i}}$$

with

$$\text{chi-square (heterogeneity)} = \Sigma\left[\frac{(O_i - E_i)^2}{V_i}\right] - \frac{[\Sigma\,(O_i - E_i)]^2}{\Sigma V_i}$$

where

$$(O_i - E_i) = a_i - \left[\frac{(a_i + b_i)}{(a_i + b_i + c_i + d_i)} \times (a_i + c_i)\right]$$

and

$$V_i = (a_i + c_i) \times \left[\frac{(a_i + b_i)}{(a_i + b_i + c_i + d_i)}\right] \times \left\{1 - \left[\frac{(a_i + b_i)}{(a_i + b_i + c_i + d_i)}\right]\right\} \times \frac{(b_i + d_i)}{(a_i + b_i + c_i + d_i - 1)}$$

Calculation of assay parameters.

$$\text{Sensitivity} = (\Sigma a_i)/(\Sigma a_i + \Sigma b_i)$$

$$\text{Specificity} = 1 - [(\Sigma c_i)/(\Sigma c_i + \Sigma d_i)]$$

$$\text{PPV} = (\Sigma a_i)/(\Sigma a_i + \Sigma c_i)$$

ACKNOWLEDGMENTS

I thank R. Danner, National Institutes of Health, Bethesda, Md., S. McGladdery and P. O'Hanley, Naval Medical Research Unit No. 2; and M. Yoshida, Jichi Medical School, Togichi, Japan; for unpublished data or the clarification of previously published data. The assistance of the staff of the medical library at Children's Hospital in accessing the literature and the comments of Jack Levin (San Francisco Veterans Affairs Hospital) and Arnold L. Smith are gratefully acknowledged.

I am currently supported by the American Cystic Fibrosis Foundation and a traveling fellowship administered by The Royal Australasian College of Physicians.

REFERENCES

1. **Adinolfi, L. E., R. Utili, G. B. Gaeta, P. Perna, and G. Ruggiero.** 1987. Presence of endotoxemia and its relationship to liver dysfunction in patients with typhoid fever. Infection **15**:359–362.
2. **Andersen, B. M., and O. Solberg.** 1984. Endotoxin liberation and invasivity of *Neisseria meningitidis*. Scand. J. Infect. Dis. **16**:247–254.
3. **Andersen, L. W., L. Baek, H. Degn, J. Lehd, M. Krasnik, and J. P. Rasmussen.** 1987. Presence of circulating endotoxins during cardiac operations. J. Thorac. Cardiovasc. Surg. **93**:115–119.
4. **Bailey, M. E.** 1976. Endotoxin, bile salts and renal function in obstructive jaundice. Br. J. Surg. **63**:774–778.
5. **Bion, J. F., I. Badger, H. A. Crosby, P. Hutchings, K.-L. Kong, J. Baker, P. Hutton, P. McMaster, J. A. Buckels, and T. S. J. Elliot.** 1994. Selective decontamination of the digestive tract reduces gram negative pulmonary colonization but not systemic endotoxemia in patients undergoing elective liver transplantation. Crit. Care Med. **22**:40–49.
6. **Bone, R. C., C. J. Fisher, T. P. Clemmer, G. J. Slotman, C. A. Metz, R. A. Balk, and the Methylprednisolone Severe Sepsis Study Group.** 1987. A controlled trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. N. Engl. J. Med. **317**:653–658.
7. **Brandtzaeg, P., K. Bryn, P. Kierulf, R. Øvstebo, E. Namork, B. Aase, and E. Jantzen.** 1992. Meningococcal endotoxin in lethal septic shock plasma studied by gas chromatography, mass-spectrometry, ultracentrifugation, and electron microscopy. J. Clin. Invest. **89**:816–823.
8. **Brandtzaeg, P., P. Kierulf, P. Gaustad, A. Skulberg, J. N. Bruun, S. Halvorsen, and E. Sorensen.** 1989. Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. J. Infect. Dis. **159**:195–204.
9. **Butler, T., W. R. Bell, J. Levin, N. N. Linh, and K. Arnold.** 1978. Typhoid fever. Studies of blood coagulation, bacteremia, and endotoxemia. Arch. Intern. Med. **138**:407–410.
10. **Butler, T., J. Levin, D. Q. Cu, and R. I. Walker.** 1973. Bubonic plague: detection of endotoxemia with the limulus test. Ann. Intern. Med. **79**:642–646.
11. **Butler, T., J. Levin, N. N. Linh, D. M. Chau, M. Adickman, and K. Arnold.** 1976. Yersinia pestis infection in Vietnam. II. Quantitative blood cultures and detection of endotoxin in the cerebrospinal fluid of patients with meningitis. J. Infect. Dis. **133**:493–499.
12. **Casey, W. F., G. J. Hauser, R. S. Hannallah, F. M. Midgley, and W. N. Khan.** 1992. Circulating endotoxin and tumour necrosis factor during pediatric cardiac surgery. Crit. Care Med. **20**:1090–1096.
13. **Clumeck, N., S. Lauwers, A. Kahn, M. Mommens, and J.-P. Butzler.** 1977. Apport du "test limule" au diagnostic des endotoxinemies et des meningites a germes Gram-negatif. Nouv. Presse Med. **6**:1451–1454.
14. **Cooperstock, M., and L. Riegle.** 1985. Plasma limulus gelation assay in infants and children: correlation with gram negative bacterial infection and evidence for "intestinal endotoxemia." Prog. Clin. Biol. Res. **189**:329–345.
15. **Danner, R. L., R. J. Elin, J. M. Hosseini, R. A. Wesley, J. M. Reilly, and J. E. Parillo.** 1991. Endotoxemia in human septic shock. Chest **99**:169–175.
16. **Danner, R. L., C. Natanson, R. J. Elin, J. M. Hosseini, S. Banks, T. J. MacVittie, and J. E. Parillo.** 1990. *Pseudomonas aeruginosa* compared with *Escherichia coli* produces less endotoxemia but more cardiovascular dysfunction and mortality in a canine model

of septic shock. Chest **98:**1480–1487.
17. **Das, J., A. A. Schwartz, and J. Folkman.** 1973. Clearance of endotoxin by platelets: role in increasing the accuracy of the Limulus gelation test and in combating experimental endotoxemia. Surgery **74:**235–240.
18. **Dofferhoff, A. S. M., V. J. J. Bom, H. G. de Vries-Hospers, J. van Ingen, J. Meer, B. P. C. Hazenberg, P. O. M. Mulder, and J. Weits.** 1992. Patterns of cytokines, plasma endotoxin, plasminogen activator inhibitor, and acute phase proteins during the treatment of severe sepsis in humans. Crit. Care Med. **20:**185–192.
19. **Dolan, S. A., L. Riegle, R. Berzofsky, and M. Cooperstock.** 1987. Clinical evaluation of the plasma chromogenic Limulus assay. Prog. Clin. Biol. Res. **231:**405–416.
20. **Elin, R. J., and J. Hosseini.** 1985. Clinical utility of the limulus amebocyte lysate (LAL) test. Prog. Clin. Biol. Res. **189:**307–324.
21. **Elin, R. J., R. A. Robinson, A. S. Levine, and S. M. Wolff.** 1975. Lack of clinical usefulness of the limulus test in diagnosis of endotoxemia. N. Engl. J. Med. **293:**521–524.
22. **EORTC Study Group.** 1987. Ceftazidime combined with a short or a long course of amikacin for empirical therapy of gram-negative bacteremia in cancer patients with granulocytopenia. N. Engl. J. Med. **317:**1692–1698.
23. **Exley, A. R., T. Leese, M. P. Holliday, R. A. Swann, and J. Cohen.** 1992. Endotoxemia and serum tumour necrosis factor as prognostic markers in severe acute pancreatitis. Gut **33:**1126–1128.
24. **Feldman, S., and T. A. Pearson.** 1974. The Limulus test and gram-negative bacillary sepsis. Am. J. Dis. Child. **128:**172–174.
24a. **Fleiss, J. L.** 1981. Combining evidence from four fold tables, p. 160–187. Statistical methods for rates and proportions, 2nd ed. John Wiley & Sons, Inc., New York.
25. **Fossard, D. P., and V. V. Kakkar.** 1974. The limulus test in experimental and clinical endotoxemia. Br. J. Surg. **61:**798–804.
26. **Függer, R., G. Hamilton, M. Rogy, F. Herbst, W. Kwasny, M. Schemper, and F. Schultz.** 1990. Prognostic significance of endotoxin determination in patients with severe intraabdominal infection. J. Infect. Dis. **161:**1314–1315.
27. **Goodman, S. N.** 1991. Have you ever meta-analysis you didn't like? Ann. Intern. Med. **114:**244–246. (Editorial.)
28. **Harris, R. I., P. C. W. Stone, and J. Stuart.** 1983. An improved chromogenic substrate endotoxin assay for clinical use. J. Clin. Pathol. **36:**1145–1149.
29. **Harthug, S., B. Bjorvatn, and B. Østerud.** 1983. Quantitation of endotoxin in blood from patients with meningococcal disease using a limulus lysate test in combination with chromogenic substrate. Infection **11:**192–195.
30. **Hass, A., M. I. Rossberg, H. L. Hodes, A. C. Hyatt, and D. S. Hodes.** 1986. Endotoxin levels in immunocompromised children with fever. J. Pediatr. **109:**265–269.
31. **Hurley, J. C.** 1994. Sepsis management and anti-endotoxin therapy after Nebacumab: a reappraisal. Drugs **47:**855–861.
32. **Hurley, J. C., F. A. Tosolini, and W. J. Louis.** 1991. Quantitative limulus lysate assay for endotoxin and the effects of plasma. J. Clin. Pathol. **44:**849–854.
32a. **Ingoldby, C. J. H., C. Skinner, and G. R. Giles.** 1986. A comparison of methods of removing inhibitors to the chromogenic limulus assay in normal and jaundiced blood. Thromb. Res. **44:**95–100.
33. **Jacob, A. I., P. K. Goldberg, N. Bloom, G. A. Degenshein, and P. J. Kozinn.** 1977. Endotoxin and bacteria in portal blood. Gastroenterology **72:**1268–1270.
34. **Jirillo, E., D. Fumarola, L. Marcuccio, L. L. Papadia, N. Pasquetto, R. Monno, and P. D. Rinaldis.** 1977. Endotossemia e malnutrizione. Minerva Pediatr. **29:**139–142.
35. **Joiner, K. A., C. H. Hammer, E. J. Brown, R. J. Cole, and M. M. Frank.** 1982. Studies on the mechanism of bacterial resistance to complement-mediated killing. I. Terminal complement components are deposited and released from *Salmonella minnesota* S218 without causing bacterial death. J. Exp. Med. **155:**797–808.
36. **Kelsey, M. C., A. P. Lipscomb, and J. M. Mowles.** 1982. Limulus amoebocyte lysate test: an aid to the diagnosis in the septic neonate? J. Infect. **4:**69–72.
37. **Klein, K., G. J. Fuchs, P. Kulapongs, G. Mertz, R. M. Suskind, and R. E. Olson.** 1988. Endotoxemia in protein-energy malnutrition. J. Pediatr. Gastroenterol. Nutr. **7:**225–228.
38. **Kuratana, M., and P. Anderson.** 1991. Host metabolites that phenotypically increase the resistance of *Haemophilus influenzae* type b to clearance mechanisms. J. Infect. Dis. **163:**1073–1079.
39. **Levin, J., T. E. Poore, N. S. Young, S. Margolis, N. P. Zauber, A. S. Townes, and W. R. Bell.** 1972. Gram-negative sepsis: detection of endotoxemia with the limulus test with studies of associated changes in blood coagulation, serum lipids and complement. Ann. Intern. Med. **76:**1–7.
40. **Levin, J., T. E. Poore, N. P. Zauber, and R. S. Oser.** 1970. Detection of endotoxin in the blood of patients with sepsis due to gram-negative bacteria. N. Engl. J. Med. **283:**1313–1316.
41. **Levin, J., P. A. Tomasulo, and R. S. Oser.** 1970. Detection of endotoxin in human blood and demonstration of an inhibitor. J. Lab. Clin. Med. **75:**903–911.
42. **Leying, H., S. Suerbaum, H.-P. Kroll, D. Stahl, and W. Opferkuch.** 1990. The capsular polysaccharide is a major determinant of serum resistance in K-1 positive blood culture isolates of *Escherichia coli*. Infect. Immun. **58:**222–227.
43. **Liao, W., and C.-H. Floren.** 1993. Hyperlipidemic response to endotoxin—a part of the host defence mechanism. Scand. J. Infect. Dis. **25:**675–682.
44. **Lumsden, A. B., J. M. Henderson, and J. Alspaugh.** 1989. Endotoxemia during percutaneous manipulation of the obstructed biliary tree. Am. J. Surg. **158:**21–24.
45. **Magliulo, E., D. Scevalo, D. Fumarola, R. Vaccaro, A. Bertotto, and S. Burberi.** 1976. Clinical experience in detecting endotoxemia with the Limulus test in typhoid fever and other Salmonella infections. Infection **4:**21–24.
45a. **Mantel, N., and W. Haenszel.** 1959. Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst. **22:**719–748.
46. **Martinez-G, L. A., R. Quintiliani, and R. C. Tilton.** 1973. Clinical experience on the detection of endotoxemia with the limulus test. J. Infect. Dis. **127:**102–105.
47. **McCartney, A. C., J. G. Banks, G. B. Clements, J. D. Sleigh, M. Tehrani, and I. M. Ledingham.** 1983. Endotoxemia in septic shock: clinical and post mortem correlations. Intensive Care Med. **9:**117–122.
48. **McGladdery, S., R. Larasati, N. Silitonga, N. Punjabi, M. Lesmana, S. Pulungsih, and P. O'Hanley.** 1993. Acute inflammatory cytokine responses in typhoid fever, abstr. 284. Abstracts of the 1993 IDSA Annual Meeting. Clin. Infect. Dis. **17:**578.
49. **Mellado, M. C., R. Rodriguez-Contreras, M. Fernandez-Crehuet, R. Lopez-Gigosos, M. D. Rodriguez, and R. Galvez-Vargas.** 1991. Endotoxin liberation by strains of *N. meningitidis* isolated from patients and healthy carriers. Epidemiol. Infect. **106:**289–295.
50. **Oberle, M. W., G. G. Graham, and J. Levin.** 1974. Detection of endotoxemia with the limulus test: preliminary studies in severely malnourished children. J. Pediatr. **85:**570–573.
51. **Opal, S. M. (Brown University).** Personal communication.
52. **Pearson, F. C., J. Dubczak, M. Weary, G. Bruszer, and G. Donohue.** 1985. Detection of endotoxin in the plasma of patients with gram-negative bacterial sepsis by the *Limulus* amoebocyte lysate assay. J. Clin. Microbiol. **21:**865–868.
53. **Peduzzi, P., C. Shatney, J. Sheagren, C. Sprung, and The Veterans Affairs Systemic Sepsis Cooperative Study Group.** 1992. Predictors of bacteremia and gram-negative bacteremia in patients with sepsis. Arch. Intern. Med. **152:**529–535.
54. **Porter, P. J., A. R. Spievack, and E. H. Kass.** 1964. Endotoxin like activity of serum from patients with severe localized infections. N. Engl. J. Med. **271:**445–447.
55. **Roantree, R. J., and L. A. Rantz.** 1960. A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. J. Clin. Invest. **39:**72–81.
56. **Rocke, D. A., S. L. Gaffin, M. T. Wells, Y. Koen, and J. G. Brock-Utine.** 1987. Endotoxemia associated with cardiopulmonary bypass. J. Thorac. Cardiovasc. Surg. **93:**832–837.
57. **Roth, R. I., F. C. Levin, and J. Levin.** 1990. Optimization of detection of bacterial endotoxin in plasma with the Limulus test. J. Lab. Clin. Med. **116:**153–161.
58. **Saubolle, M. A., and J. H. Jorgensen.** 1987. Use of the limulus amebocyte lysate test as a cost effective screen for gram negative agents of meningitis. Diagn. Microbiol. Infect. Dis. **7:**177–183.

59. **Scheifele, D. W., P. Melton, and V. Whitchelo.** 1981. Evaluation of the limulus test for endotoxemia in neonates with suspected sepsis. J. Pediatr. **98:**899–903.
60. **Scheifele, D. W., E. M. Olsen, and M. R. Pendray.** 1985. Endotoxinemia and thrombocytopenia during neonatal necrotizing enterocolitis. Am. J. Clin. Pathol. **83:**227–229.
61. **Schiller, N. L., and K. A. Joiner.** 1986. Interaction of complement with serum-sensitive and serum-resistant strains of *Pseudomonas aeruginosa*. Infect. Immun. **54:**689–694.
62. **Schlichting, E., T. Lyberg, O. Solberg, and B. M. Andersen.** 1993. Endotoxin liberation from Neisseria meningitidis correlates to their ability to induce procoagulant and fibrinolytic factors in human monocytes. Scand. J. Infect. Dis. **25:**585–594.
63. **Schulman, K. A., H. A. Glick, H. Rubin, and J. M. Eisenberg.** 1991. Cost-effectiveness of HA-1A monoclonal antibody for gram negative sepsis. JAMA **266:**3466–3471.
64. **Shenep, J. L., P. M. Flynn, F. F. Barrett, G. L. Stidham, and D. F. Westenkirchner.** 1988. Serial quantitation of endotoxaemia and bacteraemia during therapy for gram-negative bacterial sepsis. J. Infect. Dis. **157:**565–568.
65. **Stumacher, R. J., M. J. Kovnat, and W. R. McCabe.** 1973. Limitations of the usefulness of the limulus assay for endotoxin. N. Engl. J. Med. **288:**1261–1264.
66. **Togari, H., M. Mikawa, T. Iwanaga, N. Matsumoto, A. Kawase, M. Hagisawa, T. Ogino, R. Goto, I. Watanabe, H. Kito, Y. Ogawa, and Y. Wada.** 1983. Endotoxin clearance by exchange blood transfusion in septic shock neonates. Acta Paediatr. Scand. **72:**87–92.
67. **Usawattanakul, W., S. Tharavanij, and A. Limsuwan.** 1979. *Tachypleus* lysate test for endotoxin in patients with gram negative bacterial infections. Southeast Asian J. Trop. Med. Public Health **10:**13–17.
68. **van Deventer, S. J. H., H. R. Büller, J. W. ten Cate, A. Sturk, and W. Pauw.** 1988. Endotoxaemia: an early predictor of septicaemia in febrile patients. Lancet **i:**605–608.
69. **van Deventer, S. J. H., I. de Vries, L. W. Statius van Eps, W. Pauw, H. R. Büller, A. Sturk, and J. W. ten Cate.** 1987. Endotoxemia, bacteremia and urosepsis. Prog. Clin. Biol. Res. **272:**213–244.
70. **van Dissel, J. T., R. van Furth, B. A. Compier, H. D. M. Feuth, and M. Frolich.** 1993. Survival in selected patients with gram-negative sepsis after adjunctive therapy with HA-1A. Lancet **341:**959–960.
71. **Wortel, C. H., M. A. M. von der Möhlen, S. J. H. Van Deventer, C. L. Sprung, M. Jastremski, M. J. Lubbers, C. R. Smith, I. E. Allen, and J. W. ten Cate.** 1992. Effectiveness of a human monoclonal anti-endotoxin antibody (HA-1A) in gram-negative sepsis: relationship to endotoxin and cytokine levels. J. Infect. Dis. **166:**1367–1374.
72. **Yoshida, M., T. Obayashi, H. Tamura, S. Tanaka, T. Kawai, S. Sakamoto, and Y. Miura.** 1994. Diagnostic and prognostic significance of plasma endotoxin determination in febrile patients with hematological malignancies. Eur. J. Cancer **30A:**145–147.
73. **Yusuf, S., R. Peto, J. Lewis, R. Collins, and P. Sleight.** 1985. Beta-blockade during and after myocardial infarction: an overview of the randomised trials. Prog. Cardiovasc. Dis. **27:**335–371.
74. **Ziegler, E. J., C. J. Fisher, C. L. Sprung, R. C. Straube, J. C. Sadoff, G. E. Foulke, C. H. Wortel, M. P. Fink, R. P. Dellinger, N. N. H. Teng, I. E. Allen, H. J. Berger, G. L. Knatterud, A. F. LoBuglio, C. R. Smith, and the HA-1A Sepsis Study Group.** 1991. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin—a randomized, double blind, placebo-controlled trial. N. Engl. J. Med. **324:**429–436.