Circulating Endotoxin and Antiendotoxin Antibodies During Severe Sepsis and Septic Shock

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The presence of circulating endotoxin is common during sepsis but its prognostic value is poor. We hypothesized that this lack of correlation with outcome could be related in part to the presence of circulating antiendotoxin antibodies. In a 14-bed medical intensive care unit, in an 821-bed tertiary teaching hospital, we prospectively assessed endotoxin and antiendotoxin antibodies in patients with severe sepsis or septic shock. Blood samples for the determination of circulating endotoxin and antiendotoxin antibodies were drawn when severe sepsis or septic shock were diagnosed (day 0) and then on day 1, day 2, and day 4. Daily measurements of antiendotoxin antibodies did not discriminate survivors from nonsurvivors. No antibody depletion was observed. However, during follow-up, the antiendotoxin immunoglobulin (Ig)M antibody level increased among survivors but decreased among nonsurvivors (51.2 vs −44.8 MU/mL, *P* = .007). Circulating endotoxin was detectable among 9 of 17 patients on inclusion but neither the basal value nor sequential measurements correlated with outcome. These results suggest that during severe sepsis and septic shock, circulating endotoxin is a poor prognostic marker whereas the detection of an increase in IgM antiendotoxin antibody levels could identify survivors. This increase in IgM antibody levels could be attributed to a reactivation of the immune system.

EXPERIMENTAL STUDIES suggest that endotoxin is strongly involved in the pathophysiology of severe infection related to gram-negative bacteria.[1,2] However, despite accumulating evidence that endotoxin is a major determinant in systemic manifestations of sepsis, the circulating endotoxin value is correlated weakly with outcome[3-5] except during meningococcemia.[6] This lack of correlation between endotoxemia and outcome could be related in part to the poor specificity of the currently used limulus assay method. In addition, the presence in the blood stream of specific circulating antibodies could neutralize some of the effects of endotoxin.[7] Investigations evaluating antiendotoxin antibody levels and kinetics conducted among perioperative and septic situations suggested that patients with low immunoglobulin (Ig)G or IgM antiendotoxin antibody levels were more likely to develop postoperative complications,[8,9] or die during severe infection.[10,11] Strutz et al[10] observed that the median IgG antiendotoxin antibody level assessed during follow-up evaluation discriminated survivors from nonsurvivors, but no relation was established between dynamic evolution of these antibodies and outcome. In posttraumatic patients, Pape et al[12] identified a lower production of IgM and IgG antiendotoxin production shortly before death. More interestingly, these investigators identified an increase in IgM antilipopolysaccharide antibody plasma levels as predictive of survival. During sepsis, recent studies suggested that the evolution of the immune response, assessed from the properties of monocytes, could be an important tool to discriminate survivors from nonsurvivors.[13,14]

OBJECTIVES

The aim of this study was to simultaneously assess the kinetics of circulating antiendotoxin antibodies among patients with severe sepsis or septic shock and delineate the relation to outcome.

MATERIALS AND METHODS

The study was conducted in a 14-bed medical intensive care unit, in an 821-bed tertiary teaching hospital.

Patients

All consecutive patients admitted to our intensive care unit during a 6-month period for severe sepsis or septic shock were eligible. Severe sepsis was defined by culture-proven or suspected infection associated with the presence of at least 2 of the following criteria: temperature lower than 36°C or more than 38°C, heart rate greater than 90 beats/min, respiratory rate greater than 20/minute or $PaCO_2$ greater than 32 mm Hg, leukocytosis more than 12,000/mm^3 or less than 4,000/mm^3, associated with at least one of the following signs of

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acute organ dysfunction: (1) systolic blood pressure less than 90 mm Hg or a decrease in systolic blood pressure of at least 40 mm Hg from baseline, (2) acute oliguria ($<$ 30 mL/h or $<$0.7 L/d), (3) metabolic acidosis (arterial blood pH $<$ 7.36 or hyperlactatemia), (4) arterial hypoxemia (Pa_{O_2} $<$75 mm Hg or $Pa_{O_2}/Fi_{O_2} < 250$), (5) coagulopathy ($>$50% decrease in Quick's time or a platelet count less than 100×10^9), (6) acute impairment of consciousness (Glasgow Coma scale $<$14). Shock was defined as a systolic blood pressure less than 90 mm Hg for greater than 1 hour that was unresponsive to plasma expanders or required vasopressors. The severity on admission was assessed by using the Simplified Acute Physiology Score II.[15]

Patients in shock had a continuous invasive monitoring of blood pressure; patients who were not in shock were monitored with noninvasive devices.

This study was conducted according to the local Ethics Committee for Human Research. Informed consent was obtained from patients or next of kin. Small amounts of blood were used for the study (4 mL) and were obtained during collection of samples required for the severity of clinical status and standard care.

Survivors were defined as patients alive 28 days after inclusion in the study. However, the precise date of death was used to assess the mortality according to antiendotoxin antibody levels.

Data Collection Methods

As soon as the criteria of severe sepsis or septic shock were fulfilled (day 0), whole blood (4 mL) was collected from a peripheral vein and equally distributed between 2 sterile endotoxin-free plastic tubes containing sodium heparin (120 IU) (Endotube; Chromogenix, Mölndal, Sweden) and a nonsterile tube containing ethylenediaminetetraacetic acid. Further blood samples were obtained on days 1, 2, and 4. Patients could be enrolled 24 h/d because blood samples were centrifuged and stored by the duty physician.

Circulating antiendotoxin core antibody (Endo-CAb) IgG and IgM levels were assessed by using an enzyme-linked immunosorbent assay (COA-SET EndoCAb; Chromogenix). Briefly, an equimolar mixture of 4 Rc lipopolysaccharides from 4 species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, and *Salmonella typhimurium*) was coated on specific plates. A standard curve was drawn by using sera of determined IgG and IgM concentrations. Circulating IgG and IgM levels were determined with an alkaline phosphatase–conjugated goat anti-human IgG and anti-human IgM antibodies. A specific substrate (para-nitrophenylphosphate) was added. The reaction was stopped by adding 50 mL of 3 mol/L NaOH and the samples were then read with a laser nephelemetry at 405 nm. All samples were assayed twice and the intra-assay deviation was less than 8%. Test sera were compared in an enzyme-linked immunosorbent assay to a reference serum calibrated in median units/mL (MU/mL), where 100 is the median value for 1,000 healthy adults' IgG or IgM levels.[16]

Endotoxin levels were determined by the limulus assay method (Coatest Endotoxin; Chromogenix). Briefly, tubes were immediately chilled on ice and centrifuged (190 g at 4°C for 10 min). The platelet rich plasma was separated carefully and stored at −20°C until endotoxin measurement. The endotoxin concentration was derived from a previously described chromogenic end-point limulus assay.[17] The detection limit was 0.06 EU/mL (5 pg/mL). The within- and between-assay variation of the test at 100 pg/mL of platelet-rich plasma were 8% and 4%, respectively. At 10 pg/mL of platelet-rich plasma, values of 9% and 15% were obtained.

Statistical Analysis

Quantitative values are expressed as means ± 1 SD. The Mann Whitney U test was used to compare nonparametric values. Differences in individual IgM values were calculated between the latest value available and the value measured on day 0. The Kaplan Meier method was used to estimate the survival distribution according to whether this difference yielded a positive or negative value, and the Wald test was used to assess the observed survival difference. Statistical significance was set for a P value of less than .05.

RESULTS

During the study period, 17 patients with severe sepsis or septic shock (9 men; mean age, 70 ± 14 y; SAPS II, 56 ± 29) were included. Fourteen patients were in shock at inclusion. The main characteristics of the patients are shown in Table 1.

Table 1. Main Characteristics of the 17 Patients

Patients	Age	Sex	SAPS II	Shock	Primary Site of Infection	EndoCab IgM/IgG Level at Baseline	Pathogen
Survivors							
1	66	F	18	N	Catheter	145/238	*S. aureus*
2	50	M	44	Y	Pulm	197/404	*S. aureus*
3	54	F	48	Y	Pulm	153/370	*S. pneumoniae*
4	40	F	47	Y	Bacteremia	210/630	*K. pneumoniae*
5	87	F	56	Y	Gallbladder	188/552	*E. coli*
6	94	M	32	N	GI	138/144	*S. minnesota*
Nonsurvivors							
7	76	F	32	N	Urinary	380/293	*P. aeruginosa*
8	74	M	62	Y	Pulm	175/414	Not identified
9	70	F	38	Y	Pulm	242/534	*S. aureus*
10	70	M	68	Y	Pulm	163/168	*H. influenzae*
11	63	M	33	Y	Pulm	501/652	*P. aeruginosa*
12	77	M	46	Y	Catheter	192/2,500	*E. coli*
13	61	M	99	Y	Pulm	379/380	Not identified
14	75	M	56	Y	Pulm	423/162	*Branhamella catharralis*
15	69	F	79	Y	Pulm	154/146	Not identified
16	75	M	26	Y	Urinary	275/180	*P. aeruginosa*
17	85	F	110	Y	Catheter	309/143	*K. pneumoniae*

Abbreviations: SAPS II, simplified acute physiology score; F, female; M, male; Pulm, pulmonary; GI, gastrointestinal tract.

Clinical Outcome

The mortality rate assessed on day 28 was 65%. Several blood samples could not be collected from 6 patients because death occurred before day 4.

Microbiologic Analysis

Fourteen patients had bacterial infection. Ten were related to gram-negative bacteria, 4 to gram-positive strains. In 3 patients, infection was not microbiologically documented. Seven patients had bacteremia.

Antiendotoxin antibodies were detected in all plasma samples. When all patients were analyzed, mean IgG antibodies levels did not differ statistically between day 0 (468 ± 603 MU/mL) and the subsequent sampling times: 515 ± 621 MU/mL on day 1, 689 ± 690 MU/mL on day 2, and 593 ± 751 MU/mL on day 4 ($P = .4$). Likewise, mean IgM antibody levels did not differ statistically between day 0 (248 ± 121 MU/mL) and subsequent sampling times: 225 ± 102 MU/mL on day 1, 206 ± 119 MU/mL on day 2, and 255 ± 106 MU/mL on day 4 ($P = .1$). Mean antiendotoxin IgM antibody levels were always lower than mean IgG levels.

Neither static IgG nor IgM levels were predictive of survival on day 28 (data not shown). However, the absolute difference between the latest available IgM value and the value on day 0 (△ IgM) was larger among survivors (+51.2 MU/mL vs −44.8 MU/mL, $P = .007$). In fact, survival was significantly better on day 5 ($P = .01$) in patients who exhibited an increase in the IgM level (positive △ IgM) compared with patients with a decrease in the IgM level (negative △ IgM) (Fig 1). Among survivors, a positive △ IgM was observed in each of the 3 patients with a gram-positive strain and in each of the 3 patients with a gram-negative strain. Conversely, no increase in the IgG antiendotoxin levels was observed in survivors.

Endotoxin Assay

The endotoxin level was greater than the inferior limit of detection in 9 of the 17 patients on day 0 (mean, 0.07 EU ± 0.054/mL), in 11 of the 17 patients on day 1 (mean, 0.08 ± 0.07 EU/mL), in 7 of 13 patients on day 2 (mean value: 0.064 ± 0.056 EU/mL), and in 6 of 11 patients on day 4 (mean value 0.134 ± 0.256 EU/mL). No correlation was found between circulating endotoxin levels and survival. Sustained elevated values were not associated with worse prognosis. Endotoxemia was detected in gram-negative bacteria infected patients but also in every sampling of a patient with pneumonia related to *Staphylococcus aureus*. Three survivors with documented gram-negative infection had a positive limulus test at day 0 and at day 1.

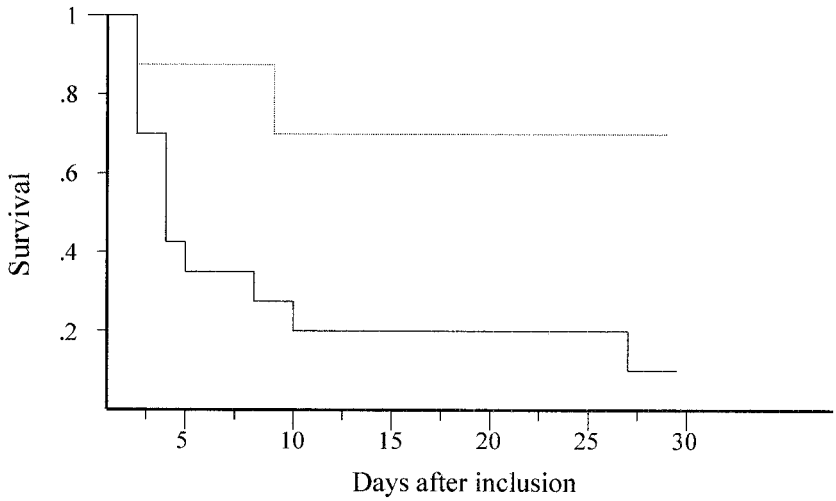

Fig 1. Survival according to time after inclusion (in days) in patients with a positive or negative △ IgM. Dotted line represents patients with a positive △ IgM; solid line represents patients with a negative △ IgM.

DISCUSSION

In this study conducted among critically ill patients with severe sepsis or sepsis shock, we observed that circulating endotoxin level was a poor prognostic marker whereas an increase in IgM antiendotoxin circulating levels was associated with survival. Although accumulating experimental data strongly suggest the key role of endotoxin in the pathophysiology of sepsis, the prognostic value of endotoxemia in clinical human sepsis is poor.[3-5] This discrepancy between experimental and clinical observations may be related to several factors. First, despite major improvements, the limulus method remains a very indirect way of measuring endotoxin. The presence in human serum of both activators and inhibitors of the chromogenic reaction and the rather cumbersome manipulations it requires make the current chromogenic method poorly suited for routine use. Moreover, the signification of a positive limulus test during authentic gram-positive–related sepsis remains questionable. Second, there are probably as many variants of endotoxin as there are gram-negative species; the endotoxin of *P. aeruginosa*, for instance, induces a stronger toxic effect than an equivalent amount of endotoxin from *E. coli*.[18] Third, natural or acquired endotoxin antagonists present in the bloodstream could inhibit the toxic effects of endotoxin to different degrees, reducing the clinical relevance of the measure of endotoxemia. We found detectable endotoxin levels in only 50% of our patients. A similar rate of endotoxin detection has been reported in severe infections.[5] Initial endotoxemia values were not predictive of outcome as previously reported.[5,18]

Antiendotoxin antibodies were detected in all the available samples. Values on day 0 were higher than those found by Goldie et al.[11] However, despite similar severity scores in the 2 studies, the latter investigators studied a very different population from ours. Forty-three percent of the patients in the study by Goldie et al[11] had intraperitoneal sepsis diagnosed at laparotomy, whereas our patients had exclusively medical pathologies. In addition, peritonitis induces a marked local immune response but a weak systemic response (assessed in terms of circulating cytokines levels),[19] suggesting that antiendotoxin antibody production also may have been compartmentalized.

We confirmed, as previously reported, the lack of correlation between static measurement of antiendotoxin antibodies and prognosis.[11] However, the pattern of IgM antibodies clearly was different between the survivors and nonsurvivors, an increase in the IgM antibody level being associated with a favorable outcome. Strutz et al[10] found that IgM concentrations in survivors and in nonsurvivors increased similarly, however, this increment was expressed in percentage and not in absolute values and concerned only the maximal values and not the last value available. Conversely, in posttraumatic patients, Pape et al[12] observed that an increase in IgM but not IgG antiendotoxin antibody levels was predictive of survival.

This increase in IgM antibodies among survivors was observed in the case of gram-positive

bacterial infections as well as in cases of gram-negative bacteria related infections. Strutz et al[10] also reported a similar pattern of antiendotoxin kinetics unrelated to whichever bacterial strain was involved (gram positive or gram negative). This point raises several questions. First, a possibly weak specificity of Endocab antibodies for endotoxin could be advocated whereas high cross reactivity to *Enterobacteriaceae* has been reported.[19,20] Second, several data suggest that, in the most severely ill patients, endotoxin could originate from the gut, induce an inflammatory response, and, finally, explain the positive limulus test in cases of severe gram-positive sepsis.[21] Third, the increase in IgM antibodies could be considered as part of a nonspecific immune system reactivation leading to a polyclonal antibody synthesis. This point is highlighted by the fact that in the present study the 3 survivors in whom endotoxin was never detected exhibited an increase in antiendotoxin IgM levels on day 4. Several arguments reinforce this hypothesis.

Munoz et al[13] in a prospective work already observed during sepsis that monocytes from surviving patients recovered their ability to produce cytokines on stimulation after a short period of hyporesponsiveness. More recent experimental and clinical data have raised the possibility of immunoparalysis during sepsis, which may resolve in survivors but persist in nonsurvivors.[14] Pape et al[12] suggested an insufficient immune defense responsible for organ dysfunction and death among posttraumatic patients.

IgM antibodies are classically the first immunoglobulin class to appear after antigenic stimulation.[22] It can be suggested that more prolonged sampling may have shown in survivors an increase in IgG antiendotoxin antibodies after the increase in IgM. This differed increase of IgG compared with IgM antibodies has been reported previously in patients with burns.[23] The hypothesis that this increase in IgM antiendotoxin antibodies could be related to a certain degree of hemoconcentration (diuretic therapy, hemodialysis) unlikely because, in this case, IgG antibodies would have been increased in survivors as well.

CONCLUSIONS

In this prospective study we observed that in patients with severe sepsis or septic shock, circulating antiendotoxin IgM antibodies kinetics could discriminate survivors from nonsurvivors. The increase in circulating antiendotoxin IgM levels observed in survivors possibly reflects a reactivation of the immune system.

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