Endotoxin Concentration in Neutropenic Patients with Suspected Gram-Negative Sepsis: Correlation with Clinical Outcome and Determination of Anti-Endotoxin Core Antibodies during Therapy with Polyclonal Immunoglobulin M-Enriched Immunoglobulins

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We carried out a study in patients with severe neutropenia from hematologic malignancy and suspected gram-negative sepsis to evaluate the clinical significance of endotoxin concentrations in plasma before and during a therapeutic intervention with a human polyclonal immunoglobulin M (IgM)-enriched immunoglobulin preparation (Pentaglobin; Biotest, Dreieich, Germany). Twenty-one patients with acute leukemia or non-Hodgkin’s lymphoma entered the study upon the development of clinical signs of gram-negative sepsis and received the IgM-enriched immunoglobulin preparation every 6 h for 3 days (total dose, 1.3 liter with 7.8 g of IgM, 7.8 g of IgA, and 49.4 g of IgG), in addition to standardized antibiotic treatment. Concentrations of endotoxin and IgM and IgG antibodies against lipid A and Re lipopolysaccharide (LPS) in plasma were determined by a modified chromogenic Limulus amebocyte lysate test and semiquantitative enzyme linked immunosorbent assay, respectively, before each immunoglobulin infusion and during the following 25 days. Seventeen patients were endotoxin positive; in five of these patients, gram-negative infection was confirmed by microbiologic findings. Prior to therapy, endotoxemia correlated significantly with the occurrence of fever, and a quantitative correlation between the endotoxin concentration and body temperature was found during the individual course of infection in 8 of the 17 patients. Overall mortality from endotoxin-positive sepsis was 41% (7 of 17) and 64% (7 of 11) in patients with symptoms of septic shock. Nonsurvivors had significantly higher maximum concentrations of endotoxin in plasma compared with those of survivors at the first study day (median of 126 versus 34 pg/ml; \[P < 0.05\]) and during the whole septic episode (median of 126 versus 61 pg/ml; \[P < 0.05\]). In survivors, immunoglobulin therapy resulted in a significant decrease in endotoxin levels in plasma within the initial 18-h treatment period, from a pretreatment median value of 28 pg/ml to a value of 8 pg/ml \([P < 0.05]\). In the seven patients who died from uncontrollable infection, no effect of therapy on endotoxin levels in plasma was observed. IgM and IgG antibodies against lipid A and Re LPS increased significantly under immunoglobulin treatment, with significant correlations between antibodies against lipid A and Re LPS. These data strongly suggest a prognostic significance of the endotoxin levels in plasma and a potential effect of treatment with a polyclonal IgM-enriched immunoglobulin preparation. Further studies are needed to substantiate these findings and to assess the impact on the clinical course by way of a prospective placebo-controlled clinical trial.

Despite the development of new and effective antimicrobial agents and their early application in combination regimens, gram-negative sepsis is still associated with an overall mortality of 20 to 40% (22, 39, 40) or even 40 to 75% in patients who develop clinical signs of septic shock (3, 16, 39, 40). This high death rate is at least partly attributed to endotoxin, the lipopolysaccharide (LPS) cell wall component of gram-negative bacteria. Endotoxin most probably exerts its toxic effects through the activation of intravascular coagulation and fibrinolysis and through triggering the complement pathways and the release of cytokines such as tumor necrosis factor and interleukins 1 and 6 from activated mononuclear cells (31, 35). Endotoxin consists of lipid A, the core region, and the side chains as its three main components. From these, lipid A is regarded as the toxic moiety (31). Lipid A and Re LPS, which comprises lipid A and a part of the inner core region, are essential for bacterial viability (31). In contrast to the antigenic diversity of the species-specific side chains, the structures of lipid A and Re LPS are highly conserved and homogeneous among different pathogenic gram-negative species, thus providing immunological cross-reactivity (31).

Antibiotic therapy alone cannot reverse evolving gram-negative septic shock because of its delayed effect on endotoxin release through inhibition of bacterial growth and cell death. Antibiotic damage of microorganisms may even temporarily increase circulating endotoxin levels and worsen the clinical course (5). Hence, neutralization of endotoxin and/or its main components has become a major target of therapeutic intervention in patients with gram-negative sepsis and has been investigated in several controlled clinical trials. In nonneutropenic patients, a murine monoclonal immunoglobulin M (IgM) antibody against lipid A (E 5) was

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found to exert beneficial effects in patients with proven gram-negative infection without clinical symptoms of shock (11). In contrast, Ziegler et al. (39) described a significant reduction in mortality after application of a human monoclonal anti-lipid A IgM antibody (HA-1A) preferentially in patients exhibiting signs of gram-negative shock. In similar patients, Schedel et al. (32) most recently reported improved survival of patients with endotoxin-positive septic shock by the administration of an IgM-enriched human polyclonal immunoglobulin preparation. These promising data warrant confirmation, the definition of possible subgroups of patients for whom the treatment would offer the greatest benefit, and especially, clarification of the underlying mechanisms.

Additional data are especially needed for patients with neutropenia, in whom the impact of endotoxin on the pathogenesis of septic shock has been controversial (12, 14, 18, 33). Also, the therapeutic role of endotoxin-neutralizing antibody preparations deserves further investigations, and beneficial responses in nonneutropenic cases may not simply be transferred to patients with severe granulocytopenia. Hence, it was the aim of the present pilot study to determine the level and kinetics of endotoxin concentrations in the plasma of neutropenic patients with suspected gram-negative sepsis in relation to the clinical course and to assess the impact of a therapeutic intervention with a human polyclonal IgM-enriched immunoglobulin preparation on the response and on the levels of endotoxin and IgM and IgG antibodies against lipid A and Re LPS in plasma.

MATERIALS AND METHODS

Patient selection. The current study was restricted to patients with severe neutropenia from underlying hematologic malignant disorders and their treatment. The patients were clinically suspected of having gram-negative sepsis or septic shock. The following patients were considered eligible for the study: (i) patients with acute myeloid or lymphoblastic leukemia, myelodysplastic syndrome, blast crisis of chronic myeloid leukemia, and non-Hodgkin's lymphoma; (ii) neutropenia (neutrophil count, <1.0×10^9/l); (iii) clinical suspicion of gram-negative sepsis or septic shock as defined by a fever of >38.5°C and/or hypotension (systolic blood pressure, <90 mm Hg), tachycardia (>100 beats per min), and shaking chills. In addition, the APACHE II score was determined to judge the clinical severity of infection on the basis of established evaluation criteria (19). Prior to therapy, patients had to give consent after having been informed about the investigational nature of the applied therapy and its potential beneficial and harmful effects. Patients younger than 18 years of age were excluded, as were patients infected with human immunodeficiency virus and patients with prior allergic reactions to immunoglobulin administration.

Therapy. All patients received a standardized antibiotic therapy according to the interventional antimicrobial strategy of the German multicenter trial of the Paul Ehrlich society, which has been described in detail elsewhere (15, 23).

Upon clinical suspicion of gram-negative sepsis or septic shock, treatment with the human polyclonal IgM-enriched immunoglobulin preparation (Pentaglobin; Biotest, Dreieich, Germany) was started. Pentaglobin is obtained from the Cohn fraction III of pooled plasma from at least 1,000 normal donors and is treated with β-propiolactone for inactivation of potentially contaminating viruses. It contains 50 g of total protein per liter and comprises approximately 6 g of IgM (12%), 6 g of IgA (12%), and 38 g of IgG (76%) per liter (34). The treatment schedule was chosen to obtain significant levels of anti-endotoxin antibodies over an extended time period, and thus, it differs from the schedule recommended by Schedel et al. (32) as follows. After an initial loading dose of 0.2 liter, patients received 0.1 liter of immunoglobulin preparation every 6 h for 72 h as slow intravenous infusions for a total dose of 1.3 liter corresponding to 7.8 g of IgM, 7.8 g of IgA, and 49.4 g of IgG.

Patients with persistent fever and/or clinical signs of sepsis were considered primary nonresponders and hence, did not qualify for a second course of immunoglobulin therapy. Patients with recurrent fever after initial defervescence were judged secondary nonresponders and received a second treatment cycle with a concomitant change of antibiotics.

Evaluation. Patients were followed for a total of 28 days after the onset of immunoglobulin treatment. Temperature, blood pressure, heart rate, clinical signs of sepsis, microbiologic findings, results of hematologic and clinical chemistry, and therapy were documented throughout the 28-day interval. Endotoxin and IgM and IgG antibodies against lipid A and Re LPS were measured immediately prior to each immunoglobulin infusion, i.e., every 6 h for 3 consecutive days, and then, for further follow-up, at days 4, 5, 6, 7, 9, 11, 14, 17, 21, and 28.

Statistical analysis. Variations of one parameter over time were evaluated by univariate repeated-measures analysis of variance, if variances were found to be homogeneous as tested by Cochran's C test and Bartlett's test (for IgM and IgG antibodies against lipid A and Re LPS, respectively). In the case of a general effect over time, values at single time points were analyzed in detail by an a posteriori comparison with the pretreatment value by one-tailed paired Student's t test, choosing an alpha value of 0.01 in order to correct for increasing type I error during multiple comparisons.

If variances were not homogeneously distributed (for endotoxin), the Friedman rank analysis of variance was applied. In the case of a general effect over time, values at single time points were analyzed in detail by comparison with the pretreatment value by the one-tailed one-many test of Nemenyi described by Miller (27) as an a posteriori test for Friedman rank analysis of variance.

Results are given as the mean ± standard error of the mean for IgM and IgG antibodies against lipid A and Re LPS, respectively, or as the median for endotoxin. Differences in a parameter between two independent groups were evaluated by the Wilcoxon rank sum test, dependencies between two parameters were evaluated by the chi-square test, and correlations were evaluated by Spearman's rank correlation coefficient.

Chromogenic Limulus amebocyte lysate test for endotoxin. A chromogenic Limulus amebocyte lysate test for endotoxin measurement was carried out as described by Schedel et al. (32), with some modifications.

After venipuncture under sterile conditions, 5 ml of blood was collected in sterile, endotoxin-free plastic tubes containing circa 25 IU of endotoxin-free sodium-heparin (Liqueminum; Hoffmann-La Roche, Grenzach, Germany) per ml. After careful mixing, the blood sample was stored for a maximum of 12 h at 4°C, and platelet-rich plasma was separated at 200 × g for 10 min at 4°C and stored at −20°C for up to 2 weeks. All reagents (Kabi Vitrum, Stockholm, Sweden) and materials were endotoxin free, and all preparation steps except for photometry (for which sterilization was not necessary because of a pH shift) were performed under sterile conditions. To minimize contamination, blood samples from healthy donors served as negative controls. A calibration
curve for endotoxin concentrations of 0, 25, 50, and 100 pg/ml was generated by adding 0, 5, 10, and 20 µl, respectively, of a standard solution containing 100 pg of endotoxin from *Escherichia coli* O111:B4 per ml (corresponding to 1.2 endotoxin units of the standard EC-5 [USP lot F U.S. Food and Drug Administration] per ml) to sterile, endotoxin-free water for a total volume of 180 µl, which was supplemented by 20 µl of endotoxin-negative platelet-rich plasma from a pool of 20 healthy donors. Correspondingly, 20 µl of patient platelet-rich plasma was diluted with 180 µl of water (or more at endotoxin concentrations of >100 pg/ml). All mixtures were heated to 75°C in a water bath for 5 min to inactivate *Limulus* amebocyte lysate inhibitors, stored at room temperature for 15 min, and shaken for 5 min to avoid attachment of endotoxin to the plastic tubes. A total of 50 µl of each sample was incubated at 37°C for 5 min in preheated (37°C) microtiter plate wells (duplicate determinations), to which 50 µl of reconstituted *Limulus* amebocyte lysate (4°C) was added (37°C for 25 min); this was followed by the addition of 100 µl of preheated (37°C) substrate buffer solution (S-2433; Ac-Ile-Glu-Gly-Arg-pNA HCl plus Tris [pH 9.0]) to each well. The *A*ₐₕᵥ was measured as soon as the lower standards of 400 U/µl showed an *A*ₐₕᵥ values of 1.5, and the results were converted into picograms of endotoxin per milliliter by linear regression analysis from the calibration curve.

The lower detection limit, which was defined as the mean absorbance ± 3 standard deviations of nine duplicate determinations of the zero value (13), was 14 pg/ml.

The intraassay (single determinations) and interassay (duplicate determinations) coefficients of variation were, respectively, 21.4 and 18.6% at a concentration of 20 pg/ml, 20.8 and 18.8% at a concentration of 50 pg/ml, and 9.9 and 8.5% at a concentration of 80 pg/ml.

**Semiquantitative enzyme-linked immunosorbent assays for IgM and IgG antibodies against lipid A and Re LPS.** Semiquantitative enzyme-linked immunosorbent assays for determination of IgM and IgG antibodies against lipid A and Re LPS were performed as described by Schedel et al. (32), with our own modifications.

The platelet-rich plasma samples were heated to 56°C in a water bath for 30 min. A calibration curve was obtained from a standard sample, which was taken from a plasma pool from 48 healthy blood donors, by six dilution steps, for which concentrations of antibodies to the endotoxin core were arbitrarily defined as 400, 200, 100, 50, 25, and 12.5 U/µl.

The antibody concentration in patient samples was determined by using fixed dilutions of 1:12,800 for IgM antibodies and 1:102,400 for IgG antibodies, which corresponds to 100 arbitrary U of the standard per µl. Patient and standard samples were diluted with phosphate-buffered saline (PBS)-Tween-4% polyethylene glycol 6000 (0.15 mol/liter containing 0.2 g of sodium azide, 0.5 ml of Tween 20 [polyoxyethylene sorbitan monolaureate], and 40 g polyethylene glycol 6000 [pH 7.4] per liter). For coating, 160 µl of sodium carbonate buffer (0.02 mol/l, pH 9.6), containing 2 µg of lipid A per ml or 2 µg of Re LPS per ml (Re LPS consists of lipid A and 3-deoxy-d-manno-octulosonic acid, a part of the inner core) isolated from *Salmonella minnesota* R595 (Sebak, Aidenbach, Germany), was placed into each well of microtiter polystyrene plates (Nunc, Roskilde, Denmark). After incubation for 24 h and three washing steps with 200 µl of PBS-Tween (150 µl of each sample was incubated for 90 min [three determinations; blank wells contained only PBS-Tween] and washing [three times]), 150 µl of rabbit anti-human IgM or rabbit anti-human IgG antiserum (Dako, Copenhagen, Denmark) labeled with alkaline phosphatase (Sigma, St. Louis, Mo.) and diluted in PBS-Tween-4% polyethylene glycol 6000 was incubated for 16 h. Samples were again washed three times, and 160 µl of 1 g of disodium p-nitrophenyl phosphate (Sigma) per liter in diethanolamine (100 g/liter) supplemented with 0.5 mmol of magnesium chloride per liter (pH 9.8) was added as the substrate. The *A*ₐₕᵥ was measured as soon as the upper standards of 400 U/µl showed an *A*ₐₕᵥ values of 1.5, and results were converted into units of antibodies to the endotoxin core per microliter by linear regression analyses from the calibration curves.

The lower detection limits, which were determined by the method described above, were 3.9 U/µl for IgM antibody against lipid A, 4.3 U/µl for IgM antibody against Re LPS, 8 U/µl for IgG antibody against lipid A, and 8.1 U/µl for IgG antibody against Re LPS.

For IgM antibody against lipid A, IgM antibody against Re LPS, IgG antibody against lipid A, and IgG antibody against Re LPS (recorded in that sequence), the intraassay (single determinations) and interassay (triple determinations) coefficients of variation were 23.7, 25.6, 20.6, and 10.9% and 20.1, 11.3, 8.7, and 25.5%, respectively, for concentrations of 12.5 U/µl; 4.8, 5.5, 16, and 8.3% and 17, 4, 14.5, and 26%, respectively, for concentrations of 100 U/µl; and 5.7, 6.3, 11.8, and 10.9% and 3.7, 13, 18, and 15.1%, respectively, for concentrations of 200 U/µl.

**RESULTS**

**Clinical course.** Twenty-one patients (14 male, 7 female) aged 20 to 75 years (median age, 56 years) from the Department of Hematology and Oncology of the University of Münster, Germany, were admitted to the study between March and October 1990. Nineteen patients entered the study once, and one patient was enrolled after two different episodes of septic shock separated by an interval of 2 months. Sixteen patients suffered from acute myeloid leukemia, one patient suffered from myelodysplastic syndrome (RAEB), one patient suffered from blast crisis of chronic myeloid leukemia, and three patients suffered from non-Hodgkin's lymphoma (two patients with centroblastic lymphoma, one patient with lymphocytic lymphoma). At the time of entry into the study, the patients had a median APACHE II score of 33 (range, 23 to 41).

Of the 21 patients, 17 had elevated endotoxin concentrations during the first day with septic symptoms and were defined as endotoxin positive. Four patients remained endotoxin negative throughout the whole clinical course. All endotoxin-negative patients recovered from the febrile episode and survived the observation period of 28 days after the initiation of immunoglobulin therapy. Of the 17 endotoxin-positive patients, 10 survived and 7 died of uncontrollable infections including disseminated intravascular coagulation with multiorgan failure (*n* = 1), pneumonia (*n* = 1), medias- tinitis (*n* = 1), urosepsis (*n* = 1), and urosepsis with acute hepatic failure (*n* = 1). According to the definition outlined above, no patient fulfilled the criterion of primary nonresponse. Four patients (three endotoxin positive, one endotoxin negative) with recurrent fever after the initial response were considered secondary nonresponders, and they received a second cycle of immunoglobulin therapy after an interval of 2 to 13 days from the first course; all four patients survived the study period.

During the study period, no adverse effects of immunoglobulin treatment were encountered.

**Endotoxin concentration and kinetics.** The measurements
TABLE 1. Differences in maximum endotoxin levels between endotoxin-positive survivors and nonsurvivors

<table>
<thead>
<tr>
<th>Study day</th>
<th>Median endotoxin level (pg/ml [range]) in:</th>
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<tbody>
<tr>
<td></td>
<td>Survivors (n = 10)</td>
</tr>
<tr>
<td>1</td>
<td>34 (15–88)*</td>
</tr>
<tr>
<td>Whole study</td>
<td>61 (18–160)*</td>
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* P < 0.05 (Wilcoxon rank sum test).

of the endotoxin levels in plasma before treatment were performed on samples collected within a median interval of 3 h (range, 0.5 to 24 h) after the onset of clinical symptoms. The pretreatment endotoxin concentrations in the plasma of the 17 endotoxin-positive patients ranged from 0 to 7.7 x 10^9 pg/ml, with a median concentration of 37 pg/ml. Maximum endotoxin levels were significantly lower in patients who survived the 28-day study period compared with those in nonsurvivors (Table 1). Setting an arbitrarily chosen limit of 90 pg/ml for the maximum endotoxin concentration during the whole study period, 9 of 11 patients with lower endotoxin levels survived, whereas 1 of 6 patients with higher endotoxin values survived.

In addition, endotoxin kinetics were found to differ substantially between survivors and nonsurvivors. In the 10 endotoxin-positive survivors, a rapid decrease in the endotoxin concentration from a median of 28 pg/ml (range, 0 to 88 pg/ml) was observed following the initiation of immunoglobulin therapy (Fig. 1). At day 5, no measurable endotoxin concentrations were detected in seven patients; in the remaining three patients endotoxin levels ranged from 20 to 31.5 pg/ml. A more detailed analysis of endotoxin kinetics by comparing the endotoxin concentrations during immunoglobulin therapy over time revealed a significant reduction in plasma endotoxin levels in relation to pretreatment concentrations as early as 18 h after the onset of immunoglobulin treatment (P < 0.05; one-tailed one-many test of Nemenyi described by Miller [27]).

The endotoxin kinetics for the seven endotoxin-positive nonsurvivors are depicted in Fig. 2. In three patients, endotoxin concentrations exceeded 10^8 pg/ml, and all three patients died from severe septic shock. Six of the seven patients died within the first 3 days during immunoglobin therapy. The seventh patient experienced an initial decrease in endotoxin levels, from 74 pg/ml before therapy to nonmeasurable concentrations at day 3; this was followed by a second increase on days 6 to 9 to 98.5 pg/ml and death on day 16. Overall, in nonsurvivors no significant decrease in endotoxin concentrations over time was observed (Friedman rank analysis of variance, P > 0.05).

Microbiologic findings and clinical symptoms. In 5 of the 17 endotoxin-positive patients, gram-negative bacteria could be identified by positive blood culture or other representative material comprising E. coli (n = 2), Pseudomonas aeruginosa (n = 2), and Enterobacter cloacae (n = 1). In the four endotoxin-negative patients, blood cultures were sterile; in one patient with meningococcal sepsis, antibodies against cytomegalovirus were identified.

All 21 patients had a fever of >38.5°C and clinical signs of sepsis or septic shock. In particular, 11 of 17 endotoxin-positive patients had or developed septic shock, which was defined by hypotension requiring vasopressor agents; 14 had tachycardia, and 6 had lactic acidosis. In contrast, one of four endotoxin-negative patients had mild hypotension without the need of vasopressor support; two patients presented with tachycardia.

The assessment of microbiologic findings and other clinical manifestations of infection revealed fever of unknown origin in six patients; four of them were endotoxin positive. Twelve patients had additional clinical symptoms, comprising pneumonia in eight patients, with microbiologic verification in five patients. Ten of these patients were endotoxin positive. Three patients had positive blood cultures only, and all three patients had increased plasma endotoxin levels. On the basis of the available microbiologic findings, the empirically chosen antimicrobial regimens were found to be adequate for survivors and nonsurvivors.

Evaluating endotoxin levels in relation to clinical symptoms, a significant correlation was found between endotoxin positivity and the occurrence of fever prior to therapy (P < 0.01; chi-square test). A quantitative relation between endotoxin levels and body temperature was not observed for the whole patient group. Analysis of the course for individual patients, however, disclosed a significant association between endotoxin levels and body temperature in 8 of 17 endotoxin-positive patients, with a median Spearman's rank correlation coefficient of 0.62.

Concentration and kinetics of IgM and IgG antibodies
against lipid A and Re LPS. IgM and IgG antibodies against lipid A and Re LPS increased significantly during immunoglobulin treatment and reached maximum levels at day 2 of therapy. A detailed analysis of the 15 patients who survived the 3-day treatment period revealed increases in IgM antibody against lipid A from a pretreatment mean of 19 U/μl to a maximum mean of 34 U/μl, IgM antibody against Re LPS from 19 up to 36 U/μl, IgG antibody against lipid A from 28 to 53 U/μl, and IgG antibody to Re LPS from 36 to 64 U/μl (Fig. 3 and 4). In nine patients, antibody concentrations could be monitored until day 17 (Fig. 5), and they showed a protracted decrease in IgM antibody against lipid A and IgM antibody against Re LPS after the last immunoglobulin infusion (by repeated-measures analysis of variance, P < 0.025 for IgM antibody against lipid A and P < 0.005 for IgM antibody against Re LPS). In contrast, IgG antibody to lipid A and IgG antibody against Re LPS showed no variation over time between the last immunoglobulin infusion and day 17 (repeated-measures analysis of variance, P > 0.05).

IgM and IgG antibodies against Re LPS reached higher concentrations than IgM and IgG antibodies against lipid A over time (Fig. 3 to 5). A significant correlation was found between IgM antibody against lipid A and IgM antibody against Re LPS prior to (r = 0.85) and after (r = 0.82) immunoglobulin treatment at day 4, and also between IgG antibody against lipid A and IgG antibody against Re LPS before (r = 0.82) and after (r = 0.87) treatment (Spearman’s rank correlation coefficients, P < 0.001).

**DISCUSSION**

The current study addressed the still pending question about the clinical significance of endotoxin determinations in patients with severe neutropenia and gram-negative sepsis and tried to elucidate the impact of a therapeutic intervention with an IgM-enriched human immunoglobulin preparation on patient outcome and levels of endotoxin and IgM and IgG antibodies against lipid A and Re LPS in plasma.

The data presented here strongly suggest a prognostic significance of endotoxin concentrations. Hence, patients who died from uncontrollable endotoxin-positive infection had significantly higher maximum plasma endotoxin levels compared with those of survivors on the first study day (median of 126 versus 34 pg/ml; P < 0.05) and during the whole septic episode (median of 126 versus 61 pg/ml; P < 0.05). In addition, endotoxin levels in survivors were significantly reduced within the first 18 h of immunoglobulin therapy, from a pretreatment median of 28 pg/ml to a level of 8 pg/ml at 18 h (P < 0.05), while they remained unchanged or even increased in nonsurvivors. A high initial endotoxin concentration was not found to be predictive of a change in the endotoxin levels during immunoglobulin therapy; however, and additional data are needed to clarify the relation and possible independent prognostic significance of both parameters. Prior to therapy, endotoxemia correlated signif-

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**FIG. 3.** Kinetics of IgM antibody concentrations against lipid A and Re LPS from 15 patients (mean ± standard error of the mean). ×, IgM to lipid A; Δ, IgM to Re LPS.

**FIG. 4.** Kinetics of IgG antibody concentrations against lipid A and Re LPS from 15 patients (mean ± standard error of the mean). *, IgG to lipid A; ▼, IgG to Re LPS.

**FIG. 5.** Kinetics of IgM (A) and IgG (B) antibody concentrations against lipid A and Re LPS from nine patients (mean ± standard error of the mean). ×, IgM to lipid A; Δ, IgM to Re LPS; *, IgG to lipid A; ▼, IgG to Re LPS.
icantly with the occurrence of fever, and a quantitative correlation between endotoxin concentration and body temperature was found in 8 of 17 individual courses of sepsis. These results are in accordance with those of prior studies in nonneutropenic patients. Hence, Van Deventer et al. (36) claimed that increased endotoxin concentrations are a better indicator of gram-negative sepsis than are positive blood cultures, and in a prospective randomized trial with an IgM-enriched immunoglobulin, Schedel et al. (32) recently reported the observation of significantly lower pretherapeutically endotoxin levels in patients who survived endotoxin-positive sepsis compared with the levels in nonsurvivors.

For patients with neutropenia, however, the data are less conclusive. In neutropenic patients, endotoxemia was associated with fever of unknown origin in 6 of 22 patients with hematologic malignancies (12) and 5 of 36 immunocompromised children (14). In contrast, Shands and McKimney (33) found no correlation between endotoxemia and the occurrence or degree of fever and suggested an impaired immunologic response to endotoxin emerging from a permanent release of endotoxin from chemotherapeutically injured gut tissues. Similarly, no association between endotoxemia and fever or between endotoxin levels and clinical outcome was found by Kinsey and Machin (18), although no details about the endotoxin assay and its precision and sensitivity were provided.

From these data it cannot be concluded, however, that endotoxin may be less pathogenic in patients with neutropenia. In animal models, neutrophil depletion prevented tumor necrosis factor-induced multiple organ damage, but it could not abolish endotoxin-associated organ toxicity (21) or oxidative stress (4). Further studies investigating endotoxin and the endotoxin-induced cascade of cytokines are needed to understand the mechanisms by which endotoxin exerts its toxic effects in patients with neutropenia and to identify relevant mediators whose neutralization, in addition to endotoxin elimination, may improve the prognosis of patients with gram-negative sepsis. In a neutropenic rat model, for example, a combined therapeutic administration of monoclonal antibodies to serotype-specific P. aeruginosa endotoxin and tumor necrosis factor provided greater protection against experimental P. aeruginosa sepsis than did either monoclonal antibody alone (28).

Further experimental studies addressed the question of which target monoclonal antibodies should be directed to and whether the unspecific indirect effects of polyclonal hyperimmunoglobulin IgM or IgG preparations might be of relevance. Investigations in neutropenic animals indicated that both the therapeutic administration of a hyperimmune IgG antibody to P. aeruginosa (29) and the therapeutic (28, 42) or prophylactic (8) application of monoclonal IgG and IgM antibodies to species-specific endotoxin side chains protected against the corresponding homologous bacteria. A monoclonal IgM antibody (HA-1A) against lipid A of the E. coli 35 mutant, consisting of lipid A and some core sugars, also showed therapeutic efficacy and reduced mortality from heterologous Pseudomonas bacteremia (41). However, the prophylactic administration of antiserum to the E. coli 35 mutant did not decrease the E. coli-induced death rate, in contrast to the prophylactic administration of antiserum to the species-specific endotoxin side chains (37). In neutropenic patients, two randomized prophylactic clinical trials with antisera to the E. coli 35 mutant did not reveal favorable clinical effects (20, 26); in one of the trials, passive immunization was obtained in only half of the patients (20).

Experimental data strongly suggest a greater antitoxic and protective effect of polyclonal IgM rather than IgG preparations (2, 10, 24). The applied IgM-enriched human polyclonal immunoglobulin preparation Pentaglobin was shown to contain antibody titers to a variety of gram-negative bacteria (34) and to protect nonneutropenic animals from endotoxemic and gram-negative bacteraemic death (2, 34). Moreover, in a therapeutic trial it reduced mortality from endotoxin-positive sepsis in nonneutropenic patients (32), and it decreased the risk of infectious death (0 of 29 versus 6 of 34 deaths) after bone marrow transplantation when it was applied prophylactically (30).

In the present study, significant increases in IgM and IgG antibodies against lipid A and Re LPS were observed during treatment with the IgM-enriched immunoglobulin. After the end of the immunoglobulin treatment period, the respective levels decreased rapidly, indicating no sustained prophylactic or therapeutic protection. However, before and still weeks after treatment, elevated anti-endotoxin core antibody titers were found, suggesting that endogenous antibody production is maintained during aplasia. In some patients, substantial differences in antibody concentrations against lipid A and Re LPS were observed. These differences might be due to variations in the target epitopes of lipid A and Re LPS that cause selective exposure of epitopes (31). Although the mechanisms by which antibody preparations exert their potential beneficial effects are still not fully elucidated, and although it is still unclear how and whether antibodies which bind to the endotoxin core in vitro can penetrate endotoxin side chains and occupy core determinants in vivo (1, 37, 38), several clinical studies strongly suggest a potential protective effect. Patients with low levels of antibodies against endotoxin core showed an increased frequency of febrile episodes (17) and a higher mortality from gram-negative (25) and endotoxin-positive (32) septic shock. Furthermore, Ziegler et al. (39) reported a reduction in mortality from gram-negative bacteraemic shock by a human monoclonal anti-lipid A IgM antibody (HA-1A). From these data, it can be speculated that treatment with the IgM-enriched immunoglobulin preparation Pentaglobin provides highly protective antibodies by increasing the levels of IgM and IgG antibodies against endotoxin core. Because Pentaglobin is prepared from pooled human plasma, it may also contain a greater amount (6) of several antibodies to the immunodominant, species-specific endotoxin side chains, which may be protective against the homologous endotoxin (1, 8, 28, 29, 37, 42).

Despite these promising data, the mortality from endotoxin-positive sepsis reached 41% (7 of 17 patients) in the present pilot study, and was even 64% in patients with symptoms of septic shock (7 of 11 patients). At first sight, these results do not compare favorably with the response rates of prior corresponding investigations (7, 9, 15) and seem to suggest a lack of therapeutic efficacy of polyclonal IgM immunoglobulin treatment. This interpretation is not justified, however, when considering the selection of patients with a highly unfavorable prognosis. Hence, 11 (65%) of the 17 endotoxin-positive patients presented with clinical symptoms of septic shock; in contrast, septic shock was present in only 12% of respective cases in the corresponding EORTC trial (9). In addition, in some patients immunoglobulin therapy was initiated later than 3 h after the onset of clinical symptoms and might therefore be considered too late and even inadequate.

The data presented here, therefore, stimulate consecutive investigations for assessment of the clinical significance of therapeutically applied polyclonal IgM-enriched immuno-
globulin in neutropenic patients with gram-negative sepsis preferentially by way of a prospective placebo-controlled randomized comparison.

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