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New Aspects in the Treatment of Gram-negative Bacteraemia and Septic Shock

Despite the development of a spectrum of new antimicrobial drugs, sepsis and septic shock have remained associated with a high mortality rate. A recent investigation reported by the Sloan Kettering Institute revealed a lethality of 31% in a group of non-neutropenic septic patients (n = 239) and a lethality of 52% in a group of patients (n = 158) with neutropenia before onset of septic complications. If more than one pathogen was found to be involved, lethality of the septic complication increased to 80%. This high lethality led to the search for additional modalities for the treatment of sepsis and septic shock during the last years.

Characteristic symptoms of infections caused by gram-negative bacteria such as *Escherichia coli, Pseudomonas* spp., *Salmonella* or *Shigella* spp. are fever, headache, hypotension, changes in leukocyte counts, diarrhea, and in severe cases shock and death may follow. These effects of gram-negative pathogens are widely independent of bacterial viability and can also be elicited by the injection of killed bacteria or of preparations of endotoxins released from the wall of gram-negative cells after cell death (1). The obvious similarity between symptoms of gram-negative bacteraemia and the effects of endotoxin has consequently led to the assumption that endotoxin liberated from the bacterial cell wall during infection must be regarded as a causative agent of certain manifestations of septicaemia and septic shock (2, 3).

The lipid A component of the lipopolysaccharide (LPS) molecule was shown to be responsible for the majority of the endotoxic activities (4–7). Lipid A expresses affinity to various humoral factors: Lipid A was shown to be capable of activating the complement cascade by the classical as well as the alternate pathway. The excessive complement activation may have a deleterious effect in gram-negative shock, especially through increased chemotaxis of polymorphonuclear leukocytes resulting in pulmonary leukostasis, leading to the acute respiratory distress syndrome (8). In this context, the liberation of anaphylatoxins may increase the inflammatory process and lead to vascular permeability disturbances. In addition, lipid A can activate factor XII (Hageman factor), a key factor for activation of the coagulation and fibrinolytic systems as well as the bradykinin system. Activation of these systems leads to disseminated intravascular coagulation with thrombosis and consumption of coagulation factors and platelets. This process again may lead to depletion of coagulation factors and to activation of the fibrinolytic system (9, 10).

The interaction of LPS with high-density lipoprotein (HDL) is of special significance (7, 11). The HDL obviously serves as a carrier for circulating LPS or lipid A and it appears to prevent the random attachment of LPS to cells and tissues. Rather, HDL may ensure a more specific transport of LPS to organs of clearance and metabolism. The most prominent organs involved in LPS accumulation are the liver and, to some extent, the spleen. To a lesser extent, LPS can also be detected in the lung. At these body sites, LPS may interact with reticuloendothelial cells, and this event is believed to play a decisive role in the mechanism of LPS action. Notably, macrophages and Kupffer cells have been shown to form and release, as a result of exposure to lipopolysaccharide, a variety of mediators that are endowed with distinct biologic activities (12). Well-studied examples of such endogenously produced mediators are endogenous pyrogen, tumor necrosis factor, glucocorticoid antagonizing factor, plasminogen activator, procoagulant activity, colony stimulating activity, interleukin-1, insulin-like activity, prostaglandine E_2 and F_2, and others (1). These mediators can induce many of the typical endotoxin effects such as fever, hypotension, and shock. *In vivo,* these factors may act locally or they may be released into the circulation and transported to distinct susceptible target cells and organs such as the kidneys, the central nervous system and the skin. Thus, host-derived endogenous mediators may be finally responsible for the mediation of various effects observed after endotoxin injection in experimental animal models or even during the septic process in man.

Gram-negative endotoxin may be introduced into the host by absorption from infected wounds, from the intestines, by the intermittent deliberation from a septic focus and by parenteral infusion of non-sterile medical devices. Since small amounts of endotoxin can cause severe clinical symptoms, the availability of a sensitive and specific assay system for gram-negative endotoxin is crucial for the diagnosis of endotoxinaemia.

During the last ten years, considerable progress has occurred in the development of techniques for detecting endotoxin in many types of different samples and body fluids. These test systems also became applicable for routine clinical use. The limulus amoebocyte lysate-(LAL-)clotting test revealed disadvantages particularly for diagnostic use in septic patients because of its activation by many substances other then endotoxin, such as colony stimulating factor or heparin derivatives. The development of chromogenic peptide substrate could improve the LAL technique, increasing its specificity and sensitivity for gram-negative endotoxin (13). The chromogenic proce-

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dure is not influenced by substances which may interfere with the gel formation (polymerization) (14). The sensitivity of the chromogenic LAL test in human sera ranges between 1 pg/ml and 10 pg/ml. Endotoxins from different bacterial strains have been found to exhibit rather different potencies of activating the LAL reagent (15). Nevertheless, because of the high sensitivity of the chromogenic LAL test, this test system makes the clinical diagnosis of endotoxinaemia possible.

The recent development of monoclonal antibodies against determinants of gram-negative lipid A by our group offers the opportunity to determine gram-negative endotoxin semiquantitatively in an indirect enzyme linked immunosorbent assay (ELISA). This assay exhibited a sensitivity of 5 to 20 ng/ml lipid-A (from *Salmonella minnesota*). Although in our hands, the sensitivity of this ELISA-test system has been shown to be approximately ten times lower than that of the chromogenic LAL test system, the combined application of both test systems with different mechanisms of action may help to ensure the assessment of gram-negative endotoxin and to obtain clinically relevant results particularly in the septicaemic patient.

The determination of specific antibodies to endotoxic components in patient sera provides information on the individual specific humoral responsiveness against these determinants before and during septic complications (16). Lipid A and, to a smaller extent, the core-polysaccharide from nearly all clinically significant gram-negative bacteria show immunological cross reactivity (7). Thus, lipid A isolated from the LPS of *S. minnesota* R 595 can be used as a test antigen to detect antibodies against endotoxic determinants from a variety of clinically important gram-negative bacteria. Our group has developed a semi-quantitative assay system for the determination of IgG- and IgM-serum antibodies to lipid A and to LPS core determinants (17). This ELISA test system only needs two hours for performance and allows for the clinically concomitant determination of antibodies specific for endotoxic determinants. Serum antibodies to lipid A and LPS core determinants could be detected in all serum samples tested from healthy adults. The binding capacity of these antibodies was found to be decreased in the sera of a group of 93 patients with multiple myeloma and secondary immunodeficiency used as a control group (17).

In a clinical study conducted by our group, 69 patients with septicaemia were examined serially for the presence of gram-negative endotoxins as well as for specific antibodies to lipid A and LPS core determinants in the patients' sera. Patients were admitted to the study if they fulfilled at least three of the following criteria: i) fever of $> 38.5°$ C; ii) evidence of a septic focus; iii) endotoxinaemia; iv) positive blood culture; v) septic shock; vi) duration of clinical symptoms of ≤ 24 hours. Examinations of serum endotoxin levels as well as of specific antibody binding capacity showed typical patterns during the first two weeks after onset of septic complications:

1. Positive endotoxin tests in the patients' sera correlated with the onset of clinical symptoms of sepsis (chi-square test $p < 0.001$).
2. Increasing ($> 50\%$ of the initial level) or unchanged ($< 50\%$ of the initial level) serum endotoxin levels were shown to be associated with a high early mortality (mortality during the first 14 days after onset of clinical symptoms of the septic process) (16/19 patients) as compared to patients exhibiting decreasing levels ($> 50\%$ of the initial level), where two of eight patients died during this period of time (chi-square-test $p < 0.01$).
3. During the septic episode only low titers of antibodies of the IgG- as well as of the IgM-type specific for gram-negative endotoxic determinants were measurable in the patients sera. The decrease of these specific antibodies showed a significant correlation with early mortality ($p < 0.01$ [anti-lipid A IgG]; $p < 0.01$ [anti-lipid A IgM]).

These serologic constellations disclosed a specific humoral immunodeficiency to endotoxic determinants during the early phase of the clinically apparent septic process with high levels of gram-negative endotoxins circulating in the peripheral blood and simultaneously low specific antibody titers of the IgG- and the IgM-types.

From these data one could conclude that passive administration of specific antibodies to endotoxic core determinants may exhibit protective effects against the harmful consequences of gram-negative endotoxin obviously responsible for the majority of relevant clinical deviations during the septic process.

Indeed, this approach has been shown to be successful in different controlled studies (17–21). First, the administration of serum from volunteers immunized with bacteria presenting, on their surface, the core determinants of endotoxin has been shown to reduce the mortality from gram-negative septic shock by 50% (22). In a prospective randomized study, 262 surgical patients were treated with normal human plasma from volunteers or with hyperimmune plasma containing elevated antibody titers against the core glycolipid of endotoxin, respectively (20). Six of 126 recipients of hyperimmune serum developed septic shock ($p = 0.04$ when compared to the incidence in the control group); two of them died from septic shock. In contrast, in the control group, 55 of 136 patients developed severe focal infections. Of these, 15 patients developed septic shock, which resulted in death in nine. In this study, protection from shock and death was most striking in the group of patients with abdominal surgery ($p = 0.001$), in whom massive microbial contamination from the gram-negative gut flora may have occurred. This protection was not seen in the group of patients with gram-positive septic shock.

We have carried out a prospective, randomized clinical trial comprising 54 patients with septic complications. Patients meeting the admission criteria shown above were randomly allocated to two groups. One group of patients received a commercially available immunoglobulin preparation (®Pentaglobin, Biotest Pharma GmbH, Frankfurt/Main, FRG) containing high titers of IgG-, IgM- and

IgA-antibodies specific for endotoxic core determinants (23). This preparation was administered i.v. at a dosage of 600 ml on the first day of treatment, followed by 300 ml each at days two and three after starting immunoglobulin treatment. The other group did not receive any immunoglobulin preparation. It has to be stressed that patients were included in this study only when they showed clinical symptoms of septicaemia for a maximum of 24 hours. Clinical care including the use of antibiotics was adjusted to clinical necessities and was found to be comparable in both groups. During the first 14 days after the beginning of clinically apparent septicaemia, death, possibly related to the septic process occurred in six of 27 patients who received immunoglobulin treatment. In contrast 18 of the 27 patients who were randomized to the control group died during the first 14 days (chi-square test: $p \leq 0.01$). It is known from various experimental systems that immunization with *E. coli* J5 vaccine does not increase complement mediated serum bacteriolytic activity against serum resistant organisms (24). J5 antisera were shown not to be potent opsonins and to only moderately enhance clearance of bacteria. This fact led to the hypothesis that preparations containing specific antibodies to LPS core determinants act principally by binding to some portion of the LPS molecule and thus sterically block lipid A from access to mediators of septic shock in blood and tissue fluids (22). Data from an animal model of endotoxin-induced septic shock by administration of supernatants of gram-negative bacterial cultures revealed that IgG- and to a higher degree, IgM-containing antibody preparations exhibited highly significant protective activity against septic shock and death (25). In addition, the significant correlation between positive or increasing endotoxic serum activity, the binding capacity of specific IgG- and IgM-antibodies to gram-negative endotoxic determinants and the prognosis of clinically apparent septic shock in our study also speaks in favour of this hypothesis. Both the IgM and IgG fractions of J5 antiserum were shown to be able to protect against lipopolysaccharide in the local Shwartzman reactions, but preliminary experiments led to the suggestion that IgM is more potent against the septic process, possibly because of its size, permiting a more efficient blockade of the lipopolysaccharide core on bacterial surfaces (22). In this context, passive administration of immunoglobulin preparations containing specific antibodies to endotoxic determinants should be performed in the very beginning of septic complications in order to neutralize gram-negative endotoxins and to prevent the harmful consequences provoked by the lipid A component. Moreover, during the later phases of the septic process, e.g. after the initiation of lipid A-induced tissue damage, no beneficial effect of immunoglobulin preparations can be expected.

The results of a study using a rabbit model of gram-negative enteric sepsis indicated that in the absence of antibiotics, the plasma level of free endotoxin is also roughly proportional to the level of bacteraemia. However, in these experiments the level of free endotoxin increased rapidly following antibiotic administration in spite of decreasing bacteraemia. In addition, although much of the liberated endotoxin in this investigation may have been derived from bacteria circulating in the peripheral blood, lysis of circulating bacteria induced by treatment with gentamicin could not entirely account for the observed increase in plasma levels of free endotoxin. Although gentamicin was successful in reducing the numbers of viable bacteria in the circulation, plasma levels of free endotoxin additionally increased eight fold. This may be due to an effect of gentamicin on bacterial cells in tissues other than the blood, or to gentamicin-induced disturbances of the gut mucosa facilitating the influx of intestinal bacteria. These experimental data provided evidence that antibiotic therapy can promote endotoxin release and may aggravate, under certain circumstances, rather than alleviate the development of shock in bacteraemic patients.

In the clinical situation, the fear of provoking shock in gram-negative bacteraemia should by no means cause the physician to hesitate using antibiotics in this life-threatening condition. In a retrospective study of more than 600 cases of gram-negative bacteraemia it could be noted that the use of appropriate antibiotics not only decreased the frequency of shock development, but also reduced mortality, when therapy was instituted after the development of shock (26, 27). Nevertheless, even under antibiotic treatment, the amount and the effects of gram-negative endotoxin liberated during the septic process does have the potential to induce or exacerbate clinical symptoms of endotoxin shock. On the other hand, the additional application of immunoglobulin preparations as an antitoxic principle of therapy in patients carefully selected by criteria used in the clinical studies mentioned and applied at the very beginning of the septic process, may enhance the effects of antibiotics and may contribute to a better prognosis and reduced lethality in patients with gram-negative septic complications.

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