

R. G. Holzheimer

The Significance of Endotoxin Release in Experimental and Clinical Sepsis in Surgical Patients – Evidence for Antibiotic-Induced Endotoxin Release?

Summary: Sepsis and peritonitis remain a serious challenge for surgical patients, despite improvement in surgical therapy and intensive care and the introduction of new powerful antibiotics. Recent *in vitro* studies revealed the potential of certain antibiotics, e.g. penicillin-binding protein (PBP) 3-specific antibiotics, to cause antibiotic-induced endotoxin release. Other types of antibiotics, e.g., PBP 2-specific antibiotics, were associated with no or less endotoxin release. Further *in vitro* experiments and investigations in animals support the hypothesis of antibiotic-induced endotoxin release, but there is little clinical evidence. The clinical significance of endotoxin is subject of open dispute with many pro's and contra's. Endotoxin, although an important trigger, may not be the only factor to induce cytokine release, e.g., peptidoglycans were able to stimulate cells to release cytokines. Gram-positive pathogens have gained more importance in clinical sepsis and may not be sufficiently reflected in current clinical studies. The hypothesis that neutralization of endotoxin and pro-inflammatory cytokines is beneficial in sepsis was seriously challenged by the results of recent clinical and experimental studies. The better understanding of mechanisms in endotoxin-induced cell activation and cell, cell-receptor and soluble receptor interactions led to new treatment options. Recent reports on the complex pathogenesis of peritonitis and the detection of pathogen-related factors with intraperitoneal immune response may have implications on clinical studies investigating the potential of new compounds and the effect of antibiotics on endotoxin release. However, only few reports are available on the clinical significance of antibiotic-induced endotoxin release, and association of endotoxin release with pathogens, mortality or alteration of physiological parameters were not observed. With regard to the particulars of these studies, e.g., a small study population or low mortality rate, mortality may not be an ideal outcome parameter for these studies. There is clinical evidence for antibiotic-induced endotoxin release. However, the need for well-designed and performed studies using newly developed monitoring devices in intensive care therapy is obvious.

Introduction

Sepsis and intraabdominal infections continue to be a challenge in hospitals despite intensive care treatment and potent antibiotics. Sepsis has a large impact on the socio-economic system in Europe and the United States. Every year 500,000 patients suffer from sepsis in the United States and similar numbers are expected for Europe. 175,000 patients die from sepsis [1]. Overall sepsis mortality is 35%; however, in surgical patients the mortality may be even higher, ranging from 40 to 70% [2]. The mortality rate in surgical patients has not changed within the last decades, despite the introduction of powerful antibiotics (e.g., β -lactam antibiotics) [3]. Surgical therapy is still the mainstay for peritonitis treatment. Antibiotics play a role as adjuvant therapy. Without source control the mortality rate in surgical patients is as high as 80–100%. Kirschner was among the first surgeons, in 1926, to demonstrate that surgical therapy with source control, debridement and intraperitoneal lavage can lower mortality to 70% [4]. It is well known that in surgical patients the immune system

may be in a critical balance [5] and outcome may be determined mainly by the patient's own immune response [6]. In patients with elective aortic aneurysm repair it was demonstrated that the immune system is challenged by minor amounts of endotoxin released after cross-clamping of the aorta [7]. In patients with severe peritonitis and a continuously challenged immune system due to infection or operation, only minor amounts of endotoxin may be needed to tip the balance to deterioration. In fact, this may be triggered by antibiotic-induced endotoxin release.

A report by Jackson and Kropp in 1992 has revealed that there may be a different endotoxin release after different antibiotics. With PBP 2-specific antibiotics, less endotoxin was detected *in vitro* than after PBP 3-specific antibiotics (e.g., cephalosporins) [8]. The significance of *in vitro* endo-

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R. G. Holzheimer, M. D., Ph. D., Klinik für Allgemeinchirurgie, Martin-Luther-Universität Halle-Wittenberg, Ernst-Grube-Str. 40, D-06097 Halle, Germany.

toxin release and the objective to neutralize endotoxin or endotoxin-induced mediators was brought up by several investigators [9–21]. Several studies performed in different animal models support the notion that antibiotics may release different amounts of endotoxin [22–27]. The interaction of penicillin-binding proteins with antibiotics is complex and may influence LPS release and clinical response. β -lactam antibiotics specifically bind to a particular PBP which may correlate with outcome. This may trigger LPS release which is time, concentration and species dependent [28–30]. The kinetics of bacterial lysis and killing correlated with LPS in some reports; however, this hypothesis is not generally accepted [31–32]. With regard to surgical patients several questions need to be addressed:

1. Is endotoxin of clinical importance?
2. Is there a difference between compartmentalized intra-abdominal infection (surgery) and systemic sepsis (ARDS, sepsis) and what does this mean for studies and treatment of patients?
3. Is the pathogen relevant to antibiotic-induced endotoxin release in clinical circumstances?
4. Can we observe clinical changes in patients with regard to different endotoxin release and what would be the clinical parameter to study?
5. How can we improve our study setup to be able to see differences?
6. What conclusion can be drawn for clinical therapy?

Pathophysiological Significance of Endotoxin

Endotoxin is known to be an important trigger for the inflammatory response in sepsis [33–35], activating macrophages and PMN to release inflammatory cytokines [36–37]. High levels of inflammatory cytokines correlated with fever and hypotension in septic patients [38] which led to the conclusion that neutralization of endotoxin or inflammatory cytokines, e.g., TNF- α , may improve the survival in septic patients [39]. However, the results of animal and clinical studies were controversial [40, 41]. In a recent publication it was doubted that endotoxin is the trigger for the systemic inflammatory response syndrome (SIRS) after injury [42]. While it is generally accepted that endotoxin is released from disintegrating cell walls of gram-negative pathogens, the clinical significance of endotoxin is linked to the presence and determination of gram-negative pathogens in isolates from septic patients. The rate of identification of gram-negative pathogens in patients with sepsis syndrome may be low [43] or, if pathogens were isolated in the peritoneal exudate in intraabdominal infections, these pathogens may not even correlate with the outcome [44]. Berger reported a correlation of endotoxemia with pulmonary and infectious complications in surgical patients [45]. In urinary tract infections the endotoxin assay was used as a sensitive method for the detection of bacterial contamination [46]. The presence of pathogens in intra-abdominal fluid was associated with high levels of endotoxin in the systemic circulation and the peritoneal exudate

[44]. Several clinical studies investigated the effect of endotoxin on cytokine release. After i.v. endotoxin administration cytokines were elevated in healthy volunteers [47]. Endotoxin, IL-6 and phospholipase A2 were considered to be valuable tools for detection of sepsis in hematological malignancies [48]. However, soluble peptidoglycans (SPG) may also be responsible for the cytokine release from monocytes and macrophages in sepsis and the concomitant clinical alterations in sepsis. It has been demonstrated that sPG interact with CD14, a monocyte surface protein which is involved in monocyte activation, suggesting that CD14 may be a key factor in cellular stimulation by bioactive components from gram-negative and gram-positive organisms [49]. It has further been noted that the activation of cells by LPS may be a concentration-dependent mechanism. Interaction of LPS at low concentrations with target cells is CD14 dependent, whereas at high LPS concentrations it is CD14 independent. This interaction may be mediated by lipopolysaccharide-binding protein (LBP) [50]. In the search for additional LPS receptors a protein has been detected on monocytes and endothelial cells binding to lipid A. Serum factors mediating the binding of lipid A to this protein were soluble CD14 and LPS-binding protein [51]. The interaction of endotoxin or lipid A with macrophages/monocytes requires specific cellular receptors but also a unique endotoxin conformation of lipid A [52]. The effect of endotoxin on cells may also depend on the activating or deactivating effects of pro- and anti-inflammatory cytokines, receptor antagonists and soluble cytokine receptors [53]. In this respect the observation that human T cells responded in a monocyte-supported manner to LPS exposure by proliferation and production of Th1-cell-derived cytokines (IFN- γ and IL-2) merits further consideration [54]. Antibodies against endotoxin and TNF- α were used in animal and clinical studies with controversial results [55]. However, recent studies suggest that the failure of these antibodies to neutralize the biological activity of LPS may be due to a low affinity of these antibodies to endotoxin [56]. In the light of new developments of endotoxin-neutralizing compounds, e.g., BPI, endotoxin analogs or cross-reactive endotoxin-neutralizing antibodies, which were protective in animal models of sepsis in combination with antibiotics, the dispute on the clinical significance of endotoxin remains open [57–58]. For the determination of the reactivity of lipid A monoclonal antibodies it may be necessary to run the mAbs in various assays. It was demonstrated that dependent on the type of assay mAbs reacted differently [59]. However, therapies to augment natural defense against endotoxin or proinflammatory cytokines may have their limitations, according to a recent report of septic intensive care patients with high circulating levels of cytokine antagonists and a relatively small proportion of patients developing endotoxin core antibody depletion [60]. Low levels of IgM EndoCAb were an important independent predictor of an adverse postoperative outcome, which supports the hypothesis that endotoxin may be a cause of postoperative morbidity [61].

Diagnostic Aspects

The method of endotoxin determination, although a crucial factor for the evaluation of clinical studies with regard to specificity and reliability of endotoxin study results, seems to be underestimated in the discussion of the clinical significance of endotoxin. In most studies commercial test kits are used which show a good performance in plasma free solutions. However, it is known that certain compounds in the blood, e.g., plasma proteins, lipids, bactericidal permeability increasing protein (BPI), may interfere with the limulus amoebocyte lysate (LAL) reaction [62]. There is evidence for additional endotoxin neutralization of antibiotics, e. g. ciprofloxacin, aminoglycosides, which may interfere with the LAL-assay [30]. Furthermore, biologically active endotoxin may be below the detection limit of the assay, but may induce an inflammatory response nevertheless. A kinetic LAL-assay with internal standardization which includes the interference of plasma compounds in the measurement may be more reliable for assessment of endotoxin [63]. This system allows the determination of endotoxin-neutralizing capacity (ENC) in the blood, a global index of the host defense system [64]. Other investigators used endotoxin core antibodies (EndoCAB) [60] or TNF- α [65] to describe the effects of endotoxin, based on the assumption that endotoxin triggers the EndoCAB and TNF- α production. Several investigators used IL-6 for evaluation of clinical sepsis and peritonitis and demonstrated a correlation of outcome and IL-6 levels [66–70]. In general, all test methods for evaluation of the clinical significance of endotoxin and the immune response in sepsis have their limitations. For clinical purposes, a test should be reliable and reproducible, easy to handle, rapid and available for a reasonable price. A new system to measure IL-6 within 70 min is currently being tested in a large European multicenter study [70].

Differences in Intraabdominal Sepsis and Systemic Sepsis

The pathogenesis of sepsis is complex. Several cell and immune compartments become activated and may influence each other. The idea to have many septic patients in a study may be intriguing with regard to time and cost. However, recent clinical trials in which the inclusion criteria did not discriminate between different forms of sepsis were hampered by the fact that intraabdominal sepsis and systemic sepsis may not follow the same rules [5]. Several studies helped us to understand this difference. The concept that TNF- α is detrimental and blocking TNF- α is beneficial, was first challenged by *Echtenacher*, who demonstrated in a model of intraabdominal infection (cecal ligation and puncture [CLP]) that the administration of anti-TNF- α antibodies increased mortality, while the addition of TNF- α reversed the trend [71]. In another animal study systemic sepsis and intraabdominal sepsis were compared. Systemic pretreatment with anti-TNF α antibodies decreased mortality following i.v. challenge with *Escherichia coli*, but was ineffective in intraabdominal sepsis [72]. The no-

Table 1: *In vitro* studies of antibiotic-induced endotoxin/cytokine release in pathogens.

Pathogens	Antibiotics	Authors
<i>Escherichia coli</i> , <i>Salmonella</i>	E5 mAb, amoxicillin, gentamicin	Seelen et al. 1995 [9]
<i>Escherichia coli</i>	Ceftazidime, ciprofloxacin, imipenem, gentamicin, polymyxin B, rBPI-21	Prins et al. 1995 [65]
<i>Escherichia coli</i>	BPI, antibacterial 15-kDa protein isoforms (p15s), defensins	Levy et al. 1995 [10]
<i>Enterobacter cloacae</i> , <i>Escherichia coli</i>	Cefotaxime, ciprofloxacin, piperacillin	Crosby et al. 1994 [11]
<i>Escherichia coli</i>	Ceftazidime, imipenem	Bucklin et al. 1994 [12]
<i>Salmonella minnesota</i>	Teicoplanin	Foca et al. 1993 [13]
<i>Escherichia coli</i>	MaB 8G9, polymyxin B	Burd et al. 1993 [14]
<i>Escherichia coli</i>	Cefuroxime, ceftazidime, aztreonam, imipenem, taurolidine	Dofferhoff et al. 1993 [15]
<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> <i>Enterobacter cloacae</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>	Aztreonam, imipenem, quinolones	Eng et al. 1993 [16]
<i>Haemophilus influenzae</i> type b	Ceftriaxone, imipenem, polymyxin B	Arditi et al. 1993 [17]
<i>Escherichia coli</i>	Gentamicin, amoxicillin, ciprofloxacin	Van den Berg et al. 1992 [18]
<i>Haemophilus influenzae</i> type b	Ampicillin, cefotaxime, amikacin	Bingen et al. 1992 [19]
<i>Pseudomonas aeruginosa</i>	Imipenem, ceftazidime 1992	Jackson and Kropp 1992 [8]
<i>Escherichia coli</i>	Imipenem, tobramycin, ceftazidime, cefuroxime, aztreonam, chloramphenicol	Dofferhoff et al. 1991 [20]
<i>Escherichia coli</i>	Amikacin, ciprofloxacin, ceftazidime, cefotaxime, aztreonam, imipenem	Simon et al. 1994 [21]

tion that intraabdominal sepsis is different from systemic sepsis is further supported by a study in which TNF, IL-1 beta and IL-6 were less increased than after systemic LPS injection. Treatment resulted in a different outcome depending on the type of infection. Pretreatment with dexamethasone, ibuprofen and L-arginine led to reduced survival; antibiotics and pentoxifylline improved survival in mice in which CLP was performed. LPS mortality was reduced with chlorpromazine and dexamethasone [73]. There is evidence that endotoxin or LPS lead to a variable challenge of the immune system – according to the type of sepsis. The pattern of intraabdominal cytokine release in secondary peritonitis and its correlation with plasma levels and outcome may have clinical relevance. In patients with secondary peritonitis peritoneal levels of endotoxin and inflammatory mediators were higher than plasma levels and remained elevated in non-survivors. During successful operative treatment mediators decreased in survivors. However, plasma levels of the same mediators were similar in survivors and non-survivors, except for IL-6. Secondary peritonitis was associated with a significant cytokine-mediated inflammatory response that is compartmentalized in the peritoneal cavity and may indicate adverse prognosis [70]. Although certain amounts of cytokines may be beneficial to the peritoneal defense mechanisms, higher levels correlate with adverse outcome. The uncontrolled compartmentalized inflammatory response may be responsible for the failure of surgical and antibiotic treatment in many patients [74]. In another recently published study it was demonstrated that the immune response may depend on the type of trauma and sepsis. A combination of trauma and sepsis (double hit) caused a hyporesponsiveness of splenic macrophages to LPS stimulation with consecutive reduced production of inflammatory cytokines compared to trauma or sepsis alone [75].

Endotoxin Release under Different Clinical Conditions

Pathogens get access to the peritoneal cavity mostly after perforation of the intestine or an infection of intraabdominal organs. In general these are constituents of the gastrointestinal flora [76]. In cases of immunosuppression there can be alterations in the quantity of pathogens isolated. Pathogens such as *Pseudomonas*, *Serratia* and *Candida* spp. may be isolated more often [77]. There were differences in pathogen distribution with regard to pathogens in nosocomial wound infections, nosocomial infections, type of operation, type of hospital setting, e.g., intensive care unit or general ward [78]. Treatment failure may be due, partially, to the presence of resistant pathogens at the site of infection [79]. The LPS-induced cytokine immune response and the bacterial surface characteristics may be important in the process of killing of invading pathogens [80]. The functional relationship between the pathogen and the immune response is not yet fully understood. The *in vitro* studies on antibiotic-induced endotoxin release investigated mainly the effect of selected pathogens on antibiotic-induced

endotoxin release. Several *in vitro* studies have revealed that endotoxin release after antibiotic administration may also be influenced by the type of pathogen used in the model (Table 1). Induction of LPS *Pseudomonas aeruginosa* cultures suggested that ceftazidime-induced filamentation released larger quantities of bioreactive endotoxin than non-filamentous fast-lysing imipenem [8]. The effect of different types and combinations of antimicrobial agents on endotoxin release from gram-negative bacteria was observed *in vitro* and *in vivo* [20]. In whole blood assays, endotoxin was higher when cells were treated with ceftazidime, ciprofloxacin than with imipenem or gentamicin [81]. The effect of imipenem on endotoxin release may also depend on the way of administration; intraperitoneal topically applied imipenem was associated with endotoxin release [82]. Crosby reported that cefotaxime, ciprofloxacin and piperacillin caused significant endotoxin release in *in vitro* cultures of *Enterobacter cloacae* and *E. coli*. Small amounts of endotoxin were released when bacteria were exposed to tobramycin [11]. It was also demonstrated that with regard to the pathogen antibiotic-induced endotoxin release may be different with the same antibiotic. In *E. coli* ceftazidime released more endotoxin than imipenem; however, in *P. aeruginosa* endotoxin release was independent of the antibiotic used [83]. It is obvious that these studies are important to detect mechanisms of antibiotic-induced endotoxin release; for clinical purposes the investigation of the effect of polymicrobial infections may be more relevant. In established peritonitis, only a limited number of pathogens has to be considered. These infections are almost always polymicrobial, containing a mixture of aerobic and anaerobic bacteria [84–87]. Schöffel et al. [44] suggest that pathogens and respective antibiotic treatment may not influence the outcome in peritonitis.

Relevance of Pathogens in Peritonitis/Intraabdominal Infection and their Relationship to Antibiotic-Induced Endotoxin Release

In many *in vitro* and animal studies the effects of antibiotic-induced endotoxin release have been demonstrated. In selected patient groups with urosepsis or meningitis a different endotoxin release after PBP 2-specific and PBP 3-specific antibiotics was observed [88–90]. In surgical patients intraabdominal infections remain a serious challenge and antibiotics play an important role in the treatment strategy.

At the present time there are only few clinical studies investigating antibiotic-induced endotoxin release in surgical patients [91, 92]. In a retrospective analysis of data from a study with IFN- γ , Mock and coworkers found evidence that antibiotics, known to cause a release of larger amounts of endotoxin, were associated with higher TNF- α levels and a higher mortality in septic trauma patients. However, because no endotoxin was determined it may be difficult to correlate the antibiotic treatment to endotoxin release. Significantly more endotoxin-positive results after PBP

3-specific antibiotics than after imipenem were detected in surgical intensive care patients. Different PBP 3-specific antibiotics may be associated with a different kinetics of endotoxin release [92]. To quantify the amount of circulating endotoxin released following administration may not be accurate in clinical circumstances. Presently it is not known which form of endotoxin (free or neutralized, protein-bound, bacteria-bound) may activate immunocompetent cells [93]. *Brandenburg et al.* conclude that the basic determinant for endotoxicity is the conformation of the lipid A moiety, whether in its free form or as a constituent of LPS. A prerequisite for the biological activity is the conical molecular shape which may trigger cell activation [94].

Endotoxins derived from different bacterial strains may vary in their ability to activate the limulus assay [95]. Measurable levels of endotoxin activity were higher with ceftazidime than with imipenem after treatment of *E. coli* and *P. aeruginosa* strains. However, this was not observed with *Klebsiella pneumoniae*. The LAL activation in the study of antibiotic-induced endotoxin release in surgical ICU patients could not be attributed to a single pathogen. Most of the infections were polymicrobial and the number of patients in the study was too small [92].

Endotoxin is known to cause pro-inflammatory cytokine release. IL-6 has been intensively studied in patients (e.g., trauma, sepsis, elective surgery, peritonitis). There is a growing body of evidence that IL-6 may reflect the severity of disease [96, 97]. There is also evidence that imipenem administration, which was not associated with LAL activation, may be followed by a remarkable decrease of IL-6 plasma levels, whereas the decrease after administration of PBP 3-specific antibiotics may be less prominent [92]. It is known that antibiotics have immunomodulating properties [98] and macrophage activation with IL-6 release may be a "side effect" of an antibiotic. This may explain the temporary increase of IL-6 plasma levels seen after administration of some antibiotics [92].

Clinical outcome, e.g., survival, is certainly an accurate end point. However, in most recent sepsis trials, this end point was not influenced by the treatment [99–101]. The clinical course of sepsis is very complex and intensive care treatment may have confounding effects on the outcome. Certainly the mortality rate for sepsis, on an average 35%, is not high enough for verification of a significant difference with regard to outcome in small study populations. Other parameters to be considered as study end points are morbidity, functional parameters of the cardiovascular system, and scores, e.g., Apache II and III score [102, 103]. However, in a recent pilot study in surgical intensive care patients these parameters failed to detect a difference after antibiotic-induced endotoxin release [92].

The problem in all clinical studies is to decide what assay to use, to find the best time point in the clinical course where changes in clinical parameters may be visible and what clinical parameters may be reliable for detecting the effects of endotoxin release. Time series analysis techniques may

help to overcome these difficulties and should be introduced in clinical studies [104]. An automated system which handles data from a Cobas TM analyzer may automatically analyze routine laboratory and clinical parameters, calculate automatically scores, e.g., Apache II score, and analyze various proteases (proenzymes, enzyme activators, enzyme cofactors and inhibitors) [105]. Much clinical evidence has accumulated that analysis of various proteases can provide indicators and prognostic tools for severely ill patients [106]. The proenzyme functional inhibition index may contribute information on the severity of illness [107]. It became rather obvious that with a single assay no one can evaluate the immune mechanisms in the septic patient. However, the combination of time series analysis of routine laboratory and clinical data, the proteases, together with endotoxin, endotoxin-neutralizing index, and IL-6 may allow more accurate evaluation of antibiotic-induced endotoxin release.

Consequences for Clinical Therapy

In summary, there is evidence that endotoxin is a major trigger for the inflammatory response in sepsis and trauma, which makes antibiotic-induced endotoxin release a possible candidate as a risk factor in intensive care treatment. However, the pathogenesis of sepsis and peritonitis is very complex and therefore it is a difficult task to correlate outcome or morbidity with antibiotic-induced endotoxin release. Other confounding factors are pharmacodynamics of antibiotics, the sensitivity of pathogens, and the test method available for clinical research and clinical studies. The time course of different events during intensive care treatment has to be more closely observed and with regard to organ dysfunction. The methods available can improve the evaluation of antibiotics and their potential for endotoxin release.

References

1. Stone, R.: Search for sepsis drugs goes on despite past failures. *Science* 264 (1994) 365–367.
2. Wittmann, D. H., Bansal, N., Bergstein, J. M., Wallace, J. R., Wittmann, M. M., Aprahamian, C.: Staged abdominal repair compares favourably with conventional operative therapy for intraabdominal infections when adjusting for prognostic factors with a logistic model. *Theor. Surg.* 9 (1994) 201–207.
3. Wittmann, D. H., Schein, M., Condon, R. E.: Management of secondary peritonitis. *Ann. Surg.* 224 (1996) 10–18.
4. Kirschner, M.: Die Behandlung der akuten Bauchfellentzündung. *Langenbecks Arch. Klin. Chir.* 142 (1926) 253–311.
5. Holzheimer, R. G., Cavaillon, J. M., Mosca, F., Haupt, W., Stefani, A., DiStefano, R., Torres, A., Capel, P., Aasen, A., Anderson, I., Schöffel, U.: Clinical and basic science aspects of immune pathogenesis of sepsis and peritonitis. *Med. Microbiol. Letters* 5 (1996) 386–393.
6. Michie, H. R., Wilmore, D. W.: Sepsis, signals and surgical sequelae (a hypothesis). *Arch. Surg.* 125 (1990) 531–536.
7. Holzheimer, R. G., Steinmetz, W. G., Ebentreich, U., Kauffmann, T., Jendrissek, A., Franke, S., Engermann, R., Thiede, A.: Aortic aneurysm repair may cause bacterial translocation with concomitant endotoxin release into the systemic circulation and activation of the inflammatory cascade. *J. Endotoxin Research* 1 (1994) 31.

8. Jackson, J. J., Kropp, H.: Beta-lactam antibiotic induced release of endotoxin: *in vitro* comparison of penicillin-binding protein (PBP) 2-specific imipenem and PBP 3-specific ceftazidime. *J. Infect. Dis.* 165 (1992) 1033-1041.
9. Seelen, M. A., Athanassiou, P., Lynn, W. A., Norsworthy, P., Walport, M. J., Cohen, J., Davies, K. A.: The anti-lipid A monoclonal antibody E5 binds to rough gram-negative bacteria, fixes C3, and facilitates binding of bacterial immune complexes to both erythrocytes and monocytes. *Immunology* 84 (1995) 653-661.
10. Levy, O., Oos, E., Elsbach, P., Doerfler, M. E., Lehrer, R. I., Weiss, J.: Antibacterial proteins of granulocytes differ in interaction with endotoxin. Comparison of bactericidal/permeability increasing protein, p15s, and defensins. *J. Immunol.* 154 (1995) 5403-5410.
11. Crosby, H. A., Bion, J. F., Penn, C. W., Elliott, T. S.: Antibiotic-induced release of endotoxin from bacteria *in vitro*. *J. Med. Microbiol.* 40 (1994) 23-30.
12. Bucklin, S. E., Fujihara, Y., Leeson, M. C., Morrison, D. C.: Differential antibiotic-induced endotoxin release from gram-negative bacteria. *Eur. J. Clin. Microbiol. Infect. Dis.* 13 (Suppl. 1) (1994) 43-51.
13. Foca, A., Matera, G., Berlinghieri, M. C.: Inhibition of endotoxin-induced interleukin 8 release by teicoplanin in human whole blood. *Eur. J. Clin. Microbiol. Infect. Dis.* 12 (1993) 940-944.
14. Burd, R. S., Battafarano, R. J., Cody, C. S., Farber, M. S., Ratz, C. A., Dunn, D. L.: Anti-endotoxin monoclonal antibodies inhibit secretion of tumor necrosis factor-alpha by two different mechanisms. *Ann. Surg.* 218 (3) (1993) 250-259.
15. Dofferhoff, A. S., Esselink, M. T., de Vries Hospers, H. G., van Zanten, A., Bom, V. J., Weits, J., Vellenga, E.: The release of endotoxin from antibiotic-treated *Escherichia coli* and the production of tumor necrosis factor by human monocytes. *J. Antimicrob. Chemother.* 31 (1993) 373-384.
16. Eng, R. H., Smith, S. M., Fan Havard, P., Ogbara, T.: Effect of antibiotics on endotoxin release from gram-negative bacteria. *Diagn. Microbiol. Infect. Dis.* 16 (3) (1993) 185-189.
17. Arditi, M., Kabat, W., Yogeve, R.: Antibiotic-induced bacterial killing stimulates tumor necrosis factor-alpha release in whole blood. *J. Infect. Dis.* 167 (1993) 240-244.
18. Van den Berg, C., de Neeling, A. J., Schot, C. S., Hustinx, W. N., Weimer, J., de Wildt, D. J.: Delayed antibiotic-induced lysis of *Escherichia coli in vitro* is correlated with enhancement of LPS release. *Scand. J. Infect. Dis.* 24 (1992) 619-627.
19. Bingen, E., Goury, V., Bennani, H., Lambert Zechovsky, N., Aujard, Y., Darbord, J. C.: Bactericidal activity of beta-lactams and amikacin against *Haemophilus influenzae*: effect on endotoxin release. *J. Antimicrob. Chemother.* 30 (1992) 165-172.
20. Dofferhoff, A. S., Nijland, J. H., de Vries Hospers, H. G., Mulder, P. O., Weits, J., Bom, V. J.: Effects of different types and combinations of antimicrobial agents on endotoxin release from gram-negative bacteria: an *in-vitro* and *in-vivo* study. *Scan. J. Infect. Dis.* 23 (1991) 745-754.
21. Simon, D. M., Koenig, G., Trenholme, G. M.: Differences in release of tumour necrosis factor from THP-1 cells stimulated by filtrates of antibiotic-killed *Escherichia coli*. *J. Infect. Dis.* 164 (1991) 800-802.
22. Morrison, D. C., Bucklin, S. E., Leeson, M. C., Norimatsu, M.: Contribution of soluble endotoxin released from gram-negative bacteria by antibiotics to the pathogenesis of experimental sepsis in mice. *J. Endotoxin Research* 3 (1996) 237-243.
23. Bucklin, S. E., Morrison, D. C.: Differences in therapeutic efficacy among cell wall active antibiotics in a mouse model of gram-negative sepsis. *J. Infect. Dis.* 172 (1995) 1519-1527.
24. Opal, S. M., Horn, D. L., Palardy, J. E., Parejo, N., Jhung, J., Battacharjee, A., Young, L. D.: The *in vivo* significance of antibiotic-induced endotoxin release in experimental gram-negative sepsis. *J. Endotoxin Research* 3 (1996) 245-252.
25. Cohen, J., McConnell, J. S.: Release of endotoxin from bacteria exposed to ciprofloxacin and its prevention with polymyxin B. *Eur. J. Clin. Microbiol.* 5 (1986) 13-17.
26. Artenstein, A. W., Cross, A. S.: Inhibition of endotoxin reactivity by aminoglycosides. *J. Antimicrob. Chemother.* 24 (1989) 826-828.
27. Healy, D. P., Verst-Brasch, C. L., Clendening, C. E., Neely, A. N., Holder, I. A.: Influence of drug class and dose size on antibiotic-induced endotoxin/IL-6 release and impact on efficacy of anti-endotoxin antibody. *J. Endotoxin Research* 3 (1996) 219-227.
28. Lamp, K. C., Rybak, M. J., McGrath, B. J., Summers, K. K.: Influence of antibiotic and E5 monoclonal immunoglobulin M interactions on endotoxin release from *Escherichia coli* and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 40 (1996) 247-252.
29. Morrison, D. C., Bucklin, S. E.: Evidence for antibiotic-mediated endotoxin release as a contributing factor to lethality in experimental gram-negative sepsis. *Scand. J. Infect. Dis. Suppl.* 101 (1996) 3-8.
30. Nitsche, D., Schulze, C., Oesser, S., Dalhoff, A., Sack, M.: Impact of different classes antimicrobial agents on plasma endotoxin activity. *Arch. Surg.* 131 (1996) 192-199.
31. Arditi, M., Zhou, J.: Differential antibiotic-induced endotoxin release and interleukin-6 production by human umbilical vein endothelial cells (HUVECs): amplification of the response by cocubation of HUVECs and blood cells. *J. Infect. Dis.* 175 (1997) 1255-1258.
32. Hurley, J. C.: Antibiotic-induced release of endotoxin: a reappraisal. *Clin. Infect. Dis.* 15 (1992) 840-854.
33. Casey, L. C., Balk, R. A., Bone, R. C.: Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Ann. Intern. Med.* 119 (1993) 771-778.
34. Bellomo, R.: The cytokine network in the critically ill. *Anaesth. Intensive Care* 20 (1992) 288-302.
35. Goris, R. J.: Mediators of multiple organ failure. *Intensive Care Med.* 16 (Suppl. 3) (1990) S192-196.
36. van der Poll, T., Van Deventer, S. J., ten Cate, H., Levi, M., ten Cate, J. W.: Tumor necrosis factor is involved in the appearance of interleukin-1 receptor antagonist in endotoxemia. *J. Infect. Dis.* 169 (1994) 665-667.
37. Leeson, M. C., Fujihara, Y., Morrison, D. C.: Evidence for lipopolysaccharide as the predominant proinflammatory mediator in supernatants of antibiotic-treated bacteria. *Infect. Immun.* 62 (1994) 4975-4980.
38. Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., Schein, R. M. H., Sibbald, W. J.: Definition for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 101 (1992) 1644-1655.
39. Wewers, M. D., Rinehart, J. J., She, Z. W., Herzyk, D. J., Hummel, M. M., Kinney, P. A., Davis, W. B.: Tumor necrosis factor infusions in humans prime neutrophils for hypochlorous acid production. *Am. J. Physiol.* 259 (1990) L276-282.
40. Cohen, J., Carlet, J. and the Intersept Study Group: Intersept: an international multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor- α in patients with sepsis. *Crit. Care Med.* 24 (1996) 1431-1440.
41. Morrison, D. C.: Assessment of antibiotic-mediated endotoxin release. *J. Endotoxin Research* 3 (1996) 275-279.
42. Kelly, J. L., O'Sullivan, C., O'Riordain, M., O'Riordain, D., Lyons, A., Doherty, J., Mannick, J. A., Rodrick, M. L.: Is circulating endotoxin the trigger for the systemic inflammatory response syndrome seen after injury? *Ann. Surg.* 225 (1997) 530-541.
43. Willatts, S. M., Speller, D. C., Winter, R. J.: Incidence of gram-negative bacteremia in sepsis syndrome. Implications for immunotherapy. *Anaesthesia* 49 (1994) 751-754.
44. Schöffel, U., Jacobs, E., Ruf, G., Mierswa, F., von Specht, B. U., Farthmann, E. H.: Intraperitoneal microorganisms and the severity of peritonitis. *Eur. J. Surg.* 161 (1995) 501-508.
45. Berger, D., Schmidt, U. M., Ott, S., Seidelmann, M., Martin, R., Beger, H. G.: Incidence and pathophysiological relevance of post-operative endotoxemia. *FEMS Immunol. Med. Microbiol.* 11 (1995) 285-290.
46. Berger, D., Boelke, E., Seidelmann, M., Berger, H. G.: Evaluation of endotoxemia for diagnosis of urinary tract infection after major surgical procedures. *Clin. Chim. Acta* 244 (1996) 155-190.
47. Michie, H. R., Manogue, K. R., Spriggs, D. R.: Detection of circulating tumor necrosis factor after endotoxin administration. *N. Engl. J. Med.* 318 (1988) 1481-1486.

48. Rintala, E., Pulkki, K., Mertsola, J., Nevalainen, T., Nikoskelainen, J.: Endotoxin, interleukin-6 and phospholipase A2 as markers of sepsis with hematological malignancies. *Scand. J. Infect. Dis.* 27 (1995) 39–43.
49. Weidemann, B., Schletter, J., Dziarski, R., Kusumoto, S., Stelter, F., Rietschel, E. T., Flad, H. D., Ulmer, A. J.: Specific binding of soluble peptidoglycan and muramyl dipeptide to CD14 on human monocytes. *Infect. Immun.* 65 (1997) 858–864.
50. Schromm, A. B., Brandenburg, K., Rietschel, E. T., Flad, H. D., Carroll, S. F., Seydel, U.: Lipopolysaccharide-binding protein mediates CD14-independent intercalation of lipopolysaccharide into phospholipid membranes. *FEBS Lett.* 399 (1996) 267–271.
51. Schletter, J., Brade, H., Brade, L., Kruger, C., Loppnow, H., Kusumoto, S., Rietschel, E. T., Flad, H. D., Ulmer, A. J.: Binding of lipopolysaccharide (LPS) to an 80-kilodalton membrane protein of human cells is mediated by soluble CD14 and LPS-binding protein. *Infect. Immun.* 63 (1995) 2576–2580.
52. Seydel, U., Brandenburg, K., Rietschel, E. T.: A case for an endotoxic conformation. *Prog. Clin. Biol. Res.* 388 (1994) 17–30.
53. Flad, H. D., Loppnow, H., Rietschel, E. T., Ulmer, A. J.: Agonists and antagonists for lipopolysaccharide-induced cytokines. *Immunobiology* 187 (1993) 303–316.
54. Mattern, T., Thanhauser, A., Reiling, N., Toellner, K. M., Duchrow, M., Kusumoto, S., Rietschel, E. T., Ernst, M., Brade, H., Flad, H. D.: Endotoxin and lipid A stimulate proliferation of human T cells in the presence of autologous monocytes. *J. Immunol.* 153 (1994) 2996–3004.
55. Bone, R. C., Balk, R. A., Fein, A. M., Perl, T. M., Wenzel, R. P., Reines, H. D., Quenzer, R. W., Iberti, T. J., Macintyre, N., Schein, R. M.: A second large controlled clinical study of E5, a monoclonal antibody to endotoxin: results of a prospective, multicenter, randomized controlled trial. The E5 sepsis study group. *Crit. Care Med.* 23 (1995) 994–1006.
56. Marra, M. N., Thornton, M. B., Snable, J. L., Wilde, C. G., Scott, R. W.: Endotoxin-binding and -neutralizing properties of recombinant bactericidal/permeability increasing protein and monoclonal antibodies HA-1A and E5. *Crit. Care Med.* 22 (1994) 559–565.
57. Christ, W. J., Asano, O., Robidoux, A. L., Perez, M., Wang, Y., Dubuc, G. R., Gavin, W. E., Hawkins, L. D., McGuinness, P. D., Mullarkey, M. A.: E5531, a pure endotoxin antagonist of high potency. *Science* 268 (1995) 80–83.
58. DiPadova, F. E., Brade, H., Barclay, G. R., Poxton, I. R., Liehl, E., Schuetze, E., Kocher, H. P., Ramsay, G., Schreier, M. H., McClelland, D. B.: A broadly cross-protective monoclonal antibody binding to *E. coli* and *Salmonella* lipopolysaccharides. *Infect. Immun.* 61 (1993) 3863–3872.
59. Kuhn, H. M., Brade, L., Appelmek, B. J., Kusumoto, S., Rietschel, E. T., Brade, H.: The antibody reactivity of monoclonal lipid A antibodies is influenced by the acylation pattern of lipid A and the assay system employed. *Immunobiology* 189 (1993) 457–471.
60. Goldie, A. S., Fearon, K. C., Ross, J. A., Barclay, G. R., Jackson, R. E., Grant, I. S., Ramsay, G., Blyth, A. S., Howie, J. C.: Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome. The Sepsis Intervention Group. *JAMA* 274 (1995) 172–177.
61. Bennett Guerrero, E., Ayuso, L., Hamilton Davis, C., White, W. D., Barclay, G. R., Smith P. K., King, S. A., Muhlbaier, L. H., Newman, M. F., Mythen, M. G.: Relationship of preoperative antiendotoxin core antibodies and adverse outcomes following cardiac surgery. *JAMA* 277 (1997) 646–650.
62. Urbaschek, B., Ditter, B., Becker, K. P., Urbaschek, R.: Protective effects and role of endotoxin in experimental septicemia. *Circ. Shock* 14 (1984) 209–222.
63. Urbaschek, R., Becker, K. P.: Endotoxin nachweis im Plasma: Spezifität und Aussagekraft für Entwicklung und Prognose einer Sepsis. *Infusionsther. Transfusionsmed.* 20 (Suppl. 1) (1993) 16–20.
64. Nishida, J., Ekatakin, W., McDonnell, D., Urbaschek, R., Urbaschek, B., McCuskey, R.S.: Ethanol exacerbates hepatic microvascular endotoxemia and lethality in septic mice. *Shock* 1 (1994) 413–418.
65. Prins, J. M., Kuijper, E. J., Mevissen, M. L. C. M., Speelman, P., van Deventer, S. J. H.: Release of tumor necrosis factor alpha and interleukin 6 during antibiotic killing of *Escherichia coli* in whole blood: influence of antibiotic class, antibiotic concentration, and presence of septic serum. *Infect. Immun.* 63 (1995) 2236–2242.
66. Patel, R. T., Deen, K. I., Youngs, D., Warwick, J., Keighley, M. R.: Interleukin 6 is a prognostic indicator of outcome in severe intraabdominal sepsis. *Br. J. Surg.* 81 (1994) 1306–1308.
67. de Bont, E. S., Martens, A., van Raan, J., Samson, G., Fetter, W. P., Okken, A., de Leij, L. H., Kimpfen, J. L.: Diagnostic value of plasma levels of tumor necrosis factor alpha (TNF alpha) and interleukin-6 (IL-6) in newborns with sepsis. *Acta Paediatr.* 83 (1994) 696–699.
68. Fugger, R., Zadrobilek, E., Gotzinger, P., Klimann, S., Rogy, M., Winkler, S., Andel, H., Mittelbock, M., Roth, E., Schulz, F.: Perioperative TNF alpha and IL-6 concentrations correlate with septic state, organ function, and Apache II scores in intraabdominal infection. *Eur. J. Surg.* 159 (1993) 525–529.
69. Holzheimer, R. G., Schein, M., Wittmann, D. H.: Inflammatory response in peritoneal exudate and plasma of patients undergoing planned relaparotomy for severe peritonitis. *Arch. Surg.* 130 (1995) 1314–1320.
70. Holzheimer, R. G., Cavallion, J. M., Aasen, A. O., Cainzos, M., Schöffel, U.: Clinical routine determination as part of a European Research Network. *Shock* 7 (1997) 121.
71. Echtenacher, B., Falk, W., Männel, D. N., Krammer, P. H.: Requirements of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. *J. Immunol.* 145 (1990) 3762–3766.
72. Bagby, G. J., Plessala, K. J., Wilson, L. A., Thompson, J. J., Nelson, S.: Divergent efficacy of antibody to tumor necrosis factor alpha in intravascular and peritonitis models of sepsis. *J. Infect. Dis.* 163 (1991) 83–88.
73. Villa, P., Sartor, G., Angelini, M., Sironi, M., Conni, M., Gnocchi, P., Isetta, A. M., Grau, G., Buurman, W., van Tits, L. J.: Pattern of cytokines and pharmacomodulation in sepsis induced by cecal ligation and puncture compared with that induced by endotoxin. *Clin. Diagn. Lab. Immunol.* 2 (1995) 549–553.
74. Schein, M., Wittmann, D. H., Holzheimer, R., Condon, R. E.: Hypothesis: compartmentalization of cytokines in intraabdominal infection. *Surgery* 119 (1996) 694–700.
75. Holzheimer, R. G., Molloy, R., Mendez, M. V., O'Riordain, D., Curley, P., Nestor, M., Collins, K., Saporoschetz, I., Mannick, J. A., Rodrick, M. L.: Multiple system organ failure may be influenced by macrophage hypoactivation as well as hyperactivation – importance of the double challenge. *Eur. J. Surg.* 161 (1995) 795–803.
76. Hau, T., Ahrenholz, D. H., Simmons, R. L.: Secondary bacterial peritonitis: the biological basis of treatment. *Curr. Probl. Surg.* 16 (1979) 1.
77. Hau, T., Mozes, M. F., Jonasson, O.: Peritonitis nach Nierentransplantation. *Langenbecks Arch. Chir.* 353 (1981) 269.
78. Holzheimer, R. G., Quolka, P., Pätzmann, D., Füssle, R.: Nosocomial infections in general surgery: surveillance report from a German university clinic. *Infection* 18 (1990) 219–225.
79. Christou, N. V., Turgeon, P., Wassef, R., Rotstein, O., Bohnen, J., Potvin, M.: Management of intraabdominal infections. The case for intraoperative cultures and comprehensive broad-spectrum antibiotic coverage. The Canadian Intraabdominal Infection Study Group. *Arch. Surg.* 131 (1996) 1193–1201.
80. Cross, A., Asher, L., Seguin, M., Yuan, L., Kelly, N., Hammack, C., Sadoff, J., Gemski, P.: The importance of lipopolysaccharide-initiated, cytokine-mediated host defense mechanism in mice against extraintestinally invasive *Escherichia coli*. *J. Clin. Invest.* 96 (1995) 676–686.
81. Prins, J. M., Kuijpers, E. J., Mevissen, M. L., Speelman, P., van Deventer, S. J.: Release of tumor necrosis factor alpha and interleukin 6 during antibiotic killing of *Escherichia coli* in whole blood: influence of antibiotic class, antibiotic concentration, and presence of septic serum. *Infect. Immun.* 63 (1995) 2236–2242.
82. Rosman, C., Westerveld, G. J., van Oeveren, W., Kooi, K., Bleichrodt, R. P.: Effect of intraperitoneal microbials on the con-

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- centration of bacteria, endotoxin, and tumor necrosis factor in abdominal fluid and plasma in rats. *Eur. Surg. Res.* 28 (1996) 351–360.
83. Hurley, J. C.: Antibiotic-induced release of endotoxin. A therapeutic paradox. *Drug Saf.* 12 (1995) 183–195.
84. Stone, H. H., Kolb, L. D., Geheber, C. E.: Incidence and significance of intraabdominal infections. *Ann. Surg.* 181 (1975) 705–715.
85. Lorber, B., Swenson, R. M.: The bacteriology of intraabdominal infection. *Surg. Clin. North Am.* 55 (1975) 1349–1354.
86. Rotstein, O.: Peritonitis and intraabdominal abscesses. In: *Meakins, J. L.* (ed.): *Surgical infections – diagnosis and treatment.* Scientific American Medicine Inc., New York 1994, pp. 329–351.
87. Prins, J. M., van Agtmael, M. A., Kuijper, E. J., van Deventer, S. J., Speelman, P.: Antibiotic-induced endotoxin release in patients with gram-negative urosepsis: a double-blind study comparing imipenem and ceftazidime. *J. Infect. Dis.* 172 (1995) 886–891.
88. Arditi, M., Ables, L., Yoger, R.: Cerebrospinal fluid endotoxin levels in children with *H. influenzae* meningitis before and after iv ceftriaxone. *I. Infect. Dis.* 160 (1990) 1005–1011.
89. Hurley, L. C., Louis, W. J., Tosolini, F. A., Carlin, J. B.: Antibiotic-induced release of endotoxin in chronically bacteriuric patients. *Antimicrob. Agents Chemother.* 35 (1991) 2388–2394.
90. Prins, J. M.: Antibiotic induced release of endotoxin – clinical data and human studies. *J. Endotoxin Research* 3 (1996) 269–273.
91. Mock, C. N., Jurkovich, G. J., Dries, D. J., Maier, R. V.: Clinical significance of antibiotic endotoxin releasing properties in trauma patients. *Arch. Surg.* 130 (1995) 1234–1240.
92. Holzheimer, R. G., Hirte, J. F., Reith, B., Engelhardt, W., Horak, K. H., Leppert, R., Aasen, A., Capel, P., Urbaschek, R., Karch, H., Thiede, A.: Different endotoxin release and IL-6 plasma levels after antibiotic administration in surgical intensive care patients. *J. Endotoxin Research* 3 (1996) 261–267.
93. Morrison, D. C.: Assessment of antibiotic mediated endotoxin release. *J. Endotoxin Research* 3 (1996) 173–178.
94. Brandenburg, K., Seydel, U., Schromm, A. B., Loppnow, H., Koch, M. H. J., Rietschel, E. T.: Conformation of lipid A, the endotoxic center of bacterial lipopolysaccharide. *J. Endotoxin Research* 3 (1996) 173–178.
95. Opal, S. M., Horn, J. E., Palardy, J. E., Parejo, N., Jhung, J., Battarjee, A., Young, L. D.: The *in vivo* significance of antibiotic-induced endotoxin release in experimental gram-negative sepsis. *J. Endotoxin Research* 3 (1996) 245–252.
96. Biff, W. L., Moore, E. E., Moore, F. A., Peterson, V. M.: Interleukin 6 in the injured patient – marker of injury or mediator of inflammation? *Ann. Surg.* 224 (1996) 647–664.
97. Tang, G. J., Kuo, C. D., Yen, T. C., Kuo, H. S., Chan, K. H., Yien, H. W., Lee, T. Y.: Perioperative plasma concentrations of tumor necrosis factor-alpha and interleukin-6 in infected patients. *Crit. Care Med.* 24 (1996) 423–428.
98. Wittmann, D. H.: Immunological consequences of antibiotic therapy. In: *Engemann, R., Holzheimer, R. G., Thiede, A.* (eds): *Immunology and its impact on infections in surgery.* Springer Verlag, Heidelberg Berlin New York 1995, pp. 217–223.
99. Astiz, M. E., Rackow, E. C., Still, J. G., Howell, S. T., Cato, A., Von Eschen, K. B., Ulrich, J. T., Rudbach, J. A., McMahon, G., Vargas, R.: Pretreatment of normal humans with monophosphoryl lipid A induces tolerance to endotoxin: a prospective, double-blind, randomized, controlled trial. *Crit. Care Med.* 23 (1995) 9–17.
100. Bone, R. C., Balk, R. A., Fein, A. M., Perl, T. M., Wenzel, R. P., Reines, H. D., Quenzer, R. W., Iberit, T. J., MacIntyre, N., Schein, R. M.: A second large clinical study of E5, a monoclonal antibody to endotoxin: results of a prospective, multicenter randomized, controlled trial. The E5 Sepsis Study Group. *Crit. Care Med.* 23 (1995) 994–1006.
101. Greenman, R. L., Schein, R. M., Martin, M. A., Wenzel, R. P., MacIntyre, N. R., Emmanuel, G., Chmel, H., Kohler, R. B., McCarty, M., Plouffe, J.: A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram-negative sepsis. The XOMA Sepsis Study Group. *JAMA* 266 (1991) 1097–1102.
102. Rogy, M. A., Oldenburg, H. S. A., Coyle, S., Trousdale, R., Moldawer, L. L., Lowry, S. F.: Correlation between acute physiology and chronic health evaluation (Apache) III score and immunological parameters in critically ill patients with sepsis. *Br. J. Surg.* 83 (1996) 396–400.
103. Holzheimer, R. G., Haupt, W., Thiede, A., Schwarzkopf, A.: The challenge of postoperative infections: does the surgeon make a difference? *Infect. Control Hosp. Epidemiol.* 18 (1997) 449–456.
104. Imhof, M., Bauer, M.: Time series analysis in critical care monitoring. *New Horizons* 4 (1996) 519–531.
105. Aasen, A. O., Solberg, R., Sveen, O., Gallimore, M. J., Holzheimer, R. G.: Consequences of trauma on circulating cells and the plasma cascade systems. In: *Risberg, B.*: *Trauma care – an update.* Pharmacia & Upjohn, Stockholm, Sweden 1996, 118–128.
106. Hesselvik, J. F., Blombäck, M., Brodin, B., Maller, R.: Coagulation, fibrinolysis, and kallikrein systems in sepsis: relation to outcome. *Crit. Care Med.* 17 (1989) 724–733.
107. Aasen, A. O.: The proenzyme functional inhibition index. A new parameter for the evaluation of severely injured and septic patients. *Acta Chir. Scand. Suppl.* 522 (1985) 211–233.